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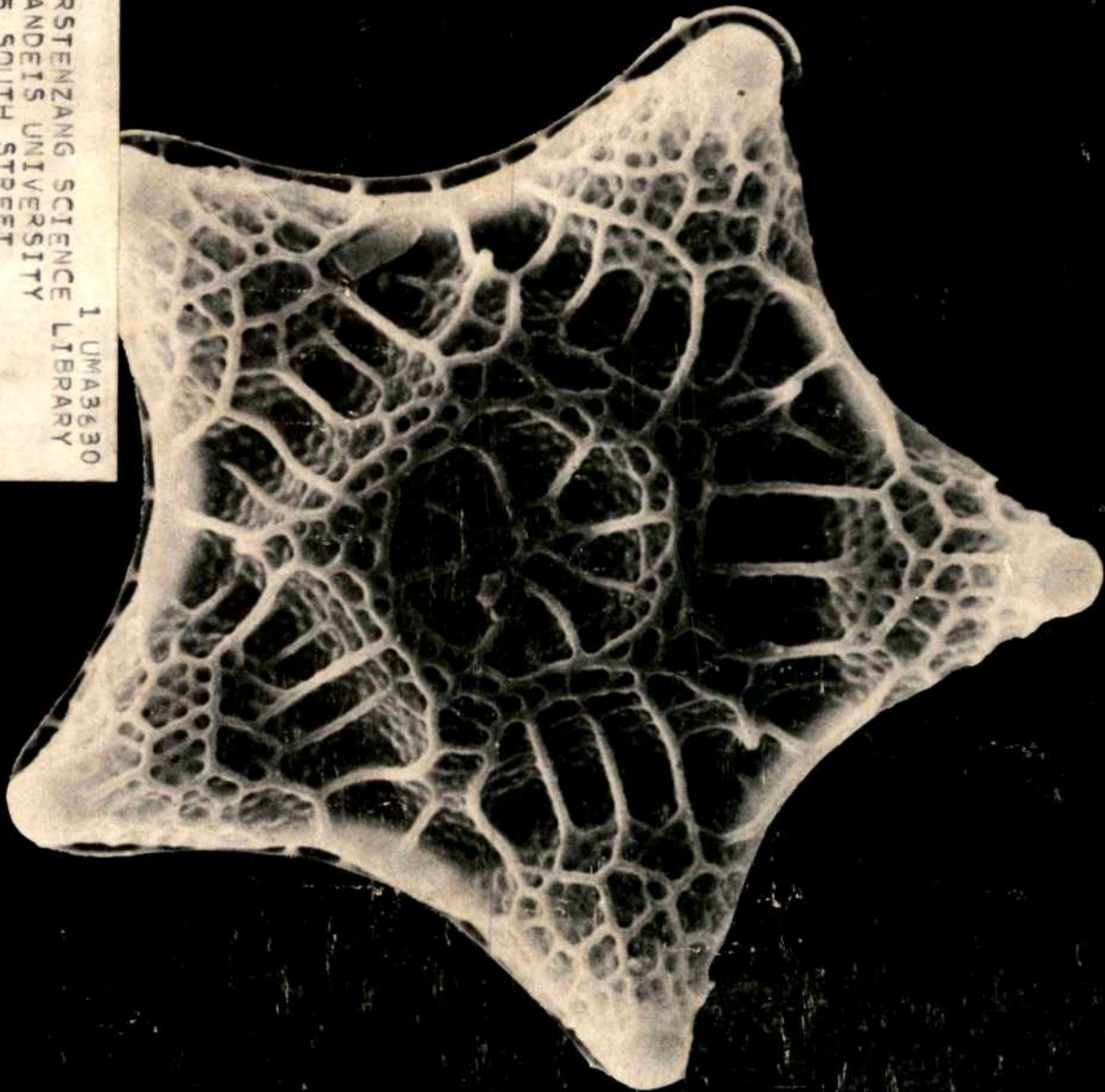
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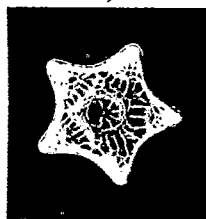
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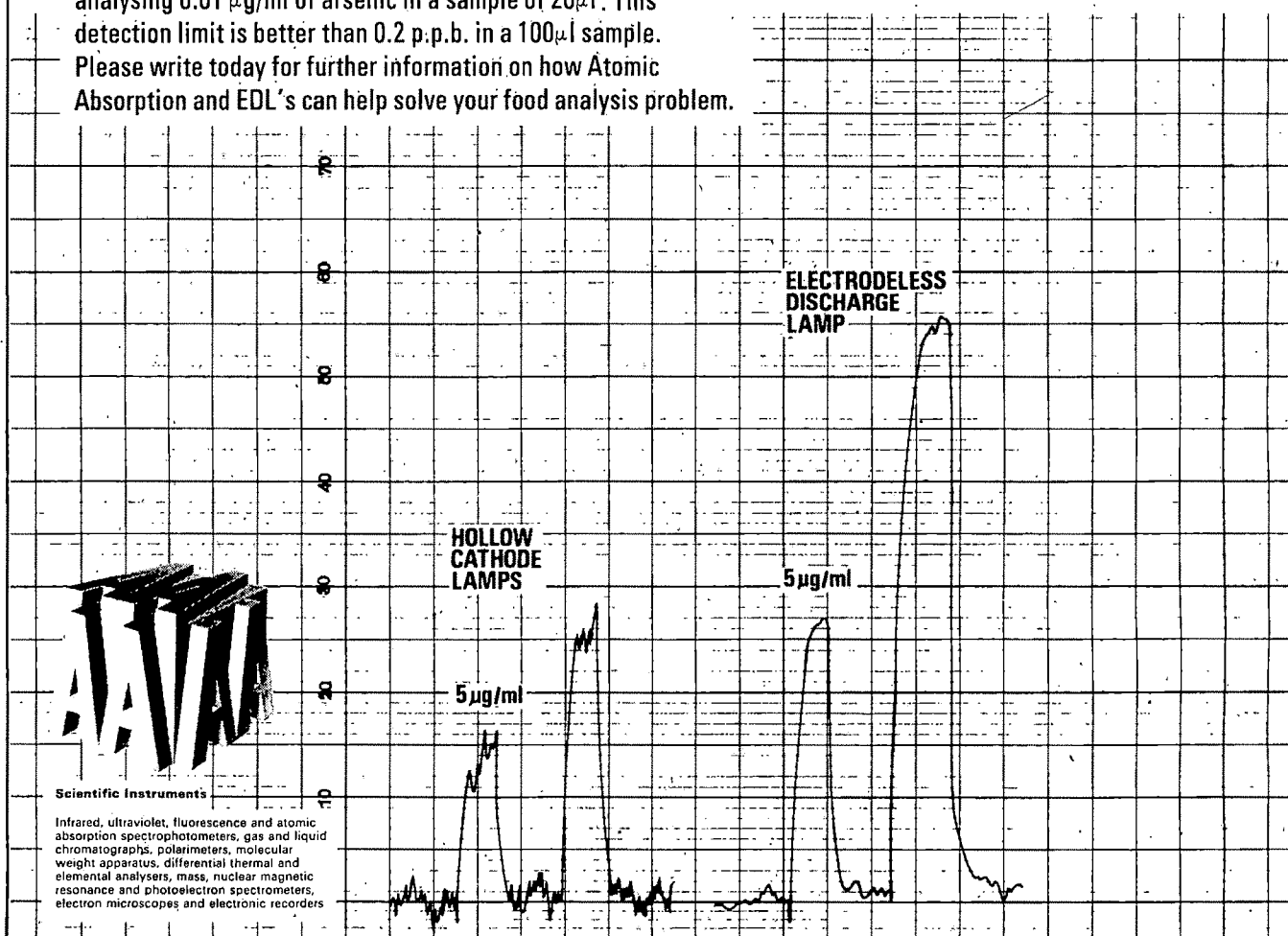
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A swinging siamang. (See page 259)

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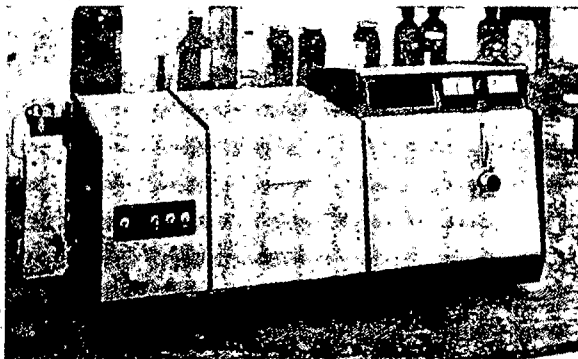
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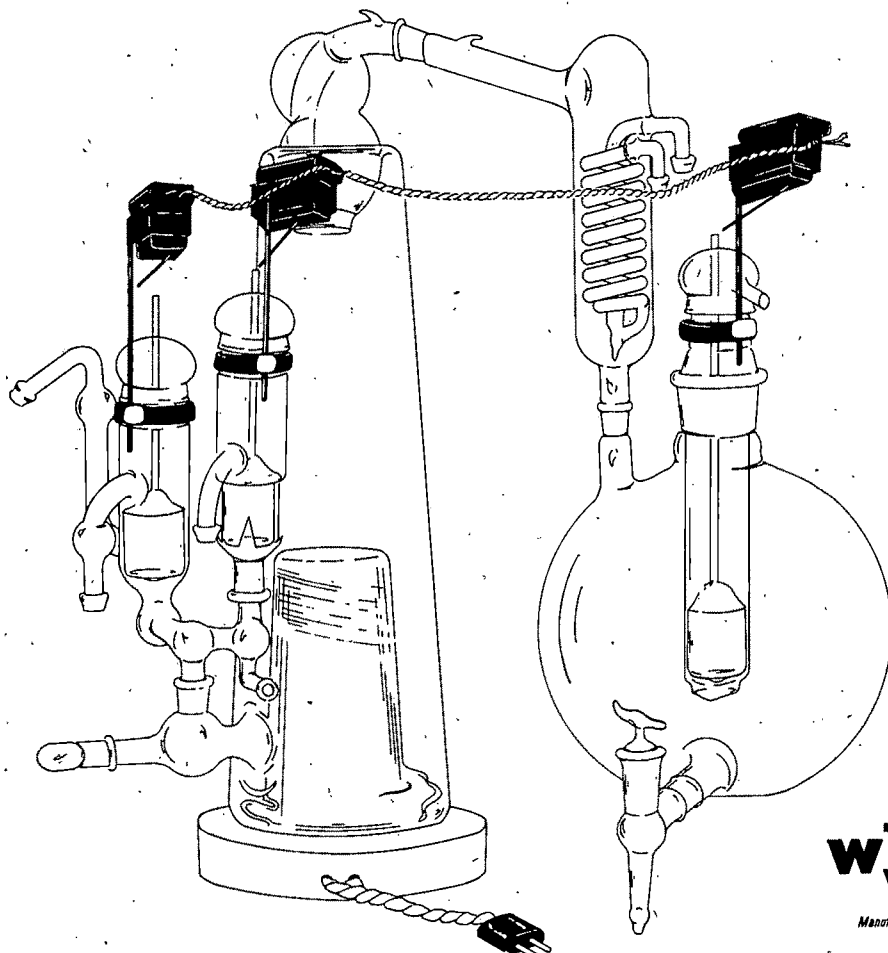
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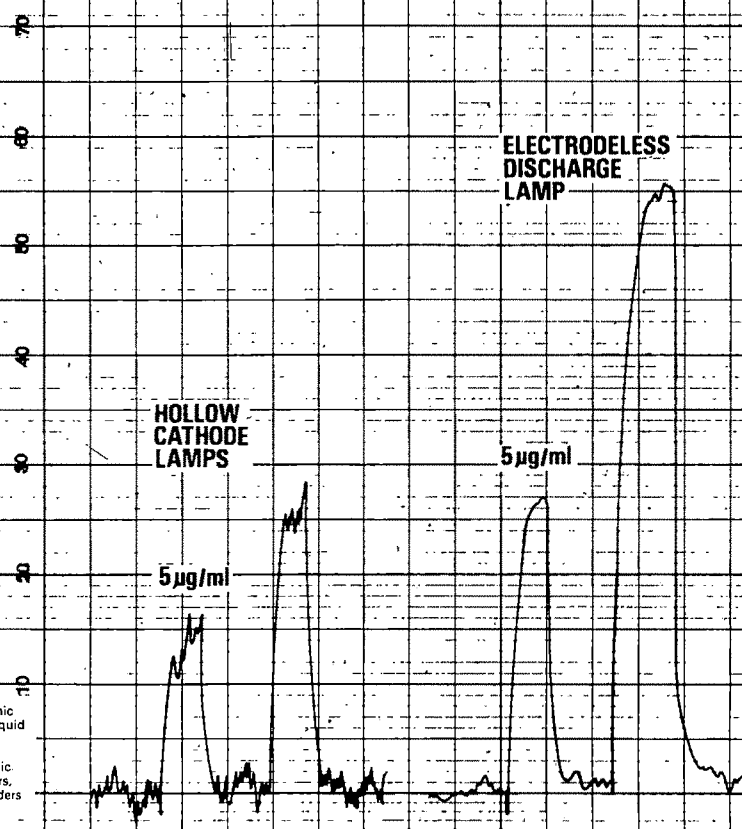
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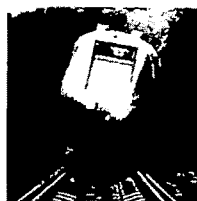
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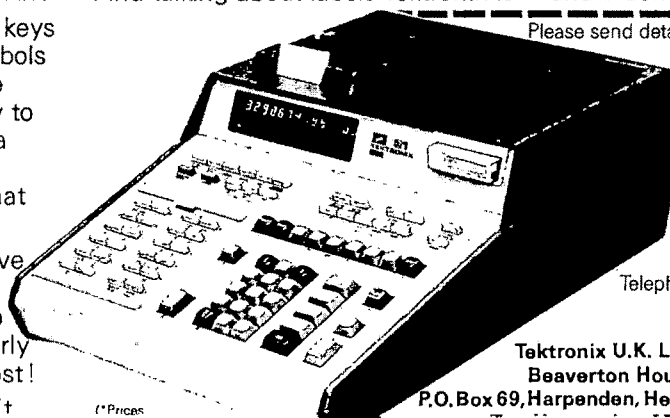
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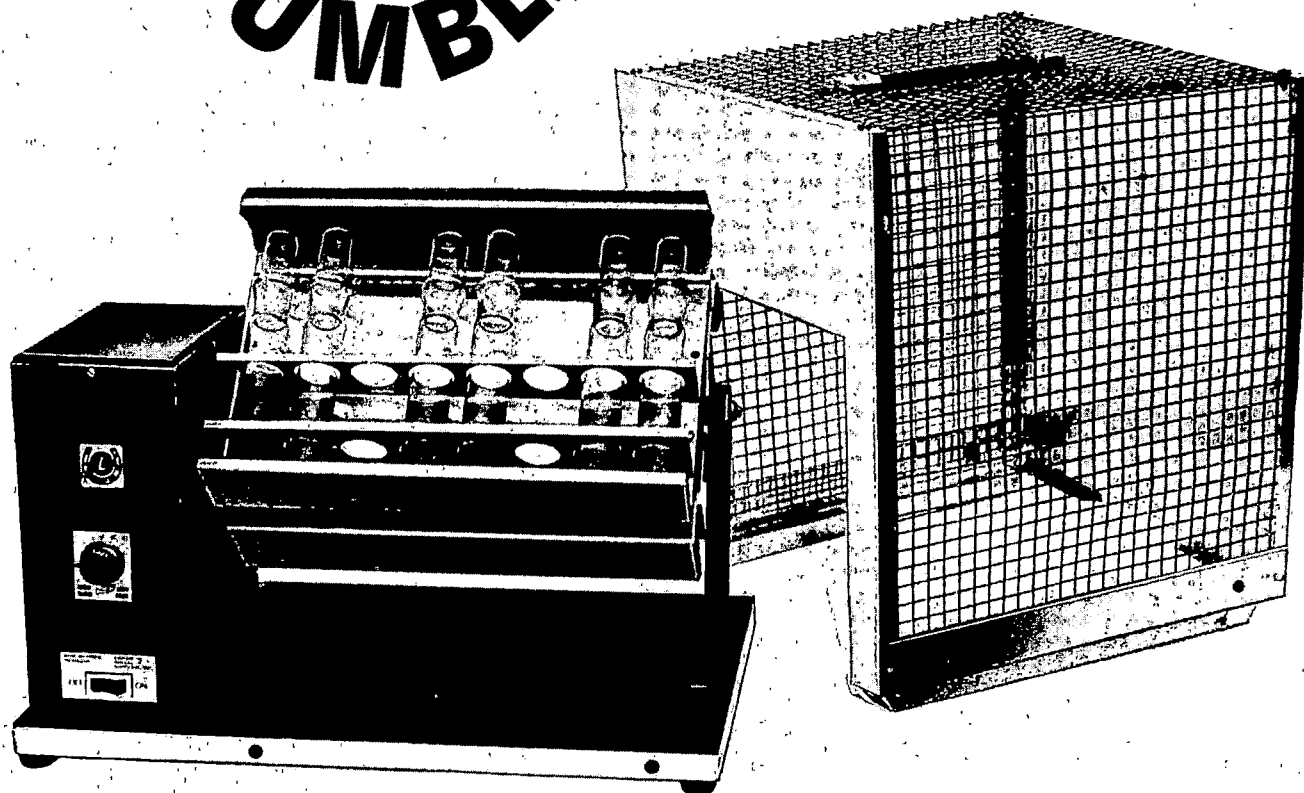
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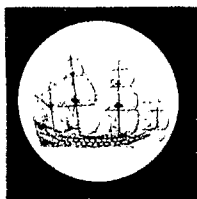
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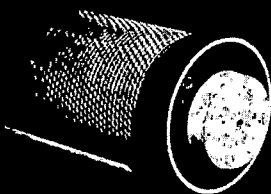
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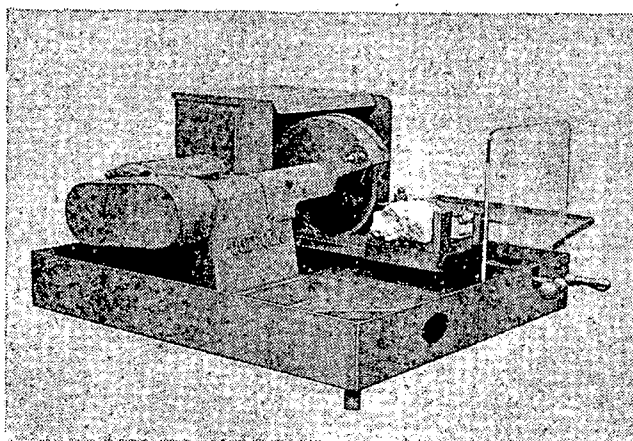


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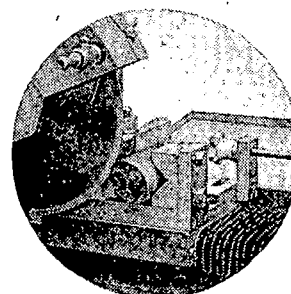
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Staff changes

THIS is the last issue for which Edward Phillips is the Physical Sciences Editor. He has been with *Nature* for six years and has built up an enviable reputation as a firm but kindly intermediary between referees and authors. He goes to a new job with the European Commission in Luxembourg.

Roger Woodham becomes Physical Sciences Editor. He did a PhD in cosmic-ray physics and has most recently been Production Editor.

Peter Newmark joins *Nature's* staff as Biological Sciences Editor. He has been a biochemist at St Bartholomew's Hospital, London and is author of a Penguin book, *Out of your mind?*

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Cover picture

Cartoon by Ray Hyden. For a more
serious view of the double helix see
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The politics of biomedical research

FOR the past eighteen months, the Nixon Administration and the biomedical research community in the United States have been engaged in a verbal battle over the management and funding of medical research. Some fundamental principles are at stake, and some deep divisions—which have resulted in the dismissal or resignation of senior officials of the National Institutes of Health—have occurred. It is important, for the health of the biomedical research effort in general, and for the future of NIH in particular, that the differences of opinion should be resolved. But how?

The nub of the dispute is this. For years, the National Institutes of Health has enjoyed considerable autonomy. It has largely been sheltered from policy decisions imposed from the White House, or from the headquarters of its parent department, the Department of Health, Education and Welfare. It has established a well-deserved international reputation, and its efforts have contributed to advances in the treatment of numerous diseases. But its budget has grown to the point at which the Office of Management and Budget is understandably asking what return the Administration is getting on its investment in biomedical research. Policy decisions vitally affecting the day-to-day operations of NIH are increasingly being made in OMB and HEW, and NIH officials and scientists therefore complain of a loss of autonomy.

Superimposed on this drift toward increased budgetary control from outside NIH is a move toward what NIH scientists refer to as 'politicalisation of research'. In the past two years, Congress has passed two bills designed to increase funding for research on cancer and diseases of the heart and lungs. The result has been that the National Cancer Institute and the National Heart and Lung Institute have both enjoyed large funding increases, while other institutes have either had their budgets cut or held constant. Moreover, an increasing proportion of the funds channelled through NIH are spent on contracts rather than the traditional grants. Contracts provide a mechanism by which research projects can be more tightly controlled. NIH scientists argue that these trends have unbalanced the overall biomedical research programme, and have taken money away from the individual university scientist—the man who has traditionally provided most of the main breakthroughs in research.

In a recent letter to the *Washington Post*, Dr Charles C. Edwards, Assistant Secretary for Health in the Department of Health, Education and Welfare, argued that NIH can no longer expect to have the autonomy it once enjoyed. Research money should be targeted on problems whose solution would have the greatest benefit for society, he argued, and the Administration has every right to expect a return on its investment.

The scientists replied that while the amount of money to be devoted to biomedical research is indeed a political question, the manner in which it is spent should be decided entirely on scientific grounds.

Both viewpoints are eminently reasonable, but can they be reconciled? Last week, Dr Edwards spoke to scientists at NIH, and according to reports, he reassured them that scientists, not politicians, would always have the final say on how research funds should be spent. Although much of his talk offered such reassurances, scientists who attended came away unconvinced that their case has really been accepted by the Administration. And their fears were fueled by the fact that the Administration's budget for 1975 carries through the trend of increasing expenditures on cancer research and heart and lung research, while holding other areas constant. Such distrust indicates the need for new planning mechanisms to be introduced for biomedical research.

Last week, Senator Edward Kennedy offered at least a step in this direction (see page 4). He has suggested that a three-member council should be set up to report directly to President Nixon on the overall balance and content of the biomedical research programme. Such a body would at least provide some insulation between NIH and the rest of the Administration. At all costs, however, the danger that it would simply turn into an outlet for the scientists' complaints, and thus generate more heat than light, must be avoided. Whether that will happen will depend to a large extent on who is appointed.

100 years ago



THE young King of Siam having come of age on October 10 last, great feasts were given to his subjects at Bangkok, the chief town of his dominion. Amongst other attractions was the ascent of a small mounted balloon, which had been constructed in Paris and had arrived by steam a few days previously. Liberal offers were made to procure an aeronaut, but were of no avail, nobody amongst the Siamese presuming to ascend. Consequently his Majesty ordered a slave, selected from amongst the less heavy of his household, to be sent up in the car. In order to encourage the poor aeronaut, so frightened for his life, he was promised to be rewarded with his enfranchisement. The ascent took place and elicited much enthusiasm from the bystanders; but, unhappily, nothing was heard from the poor fellow or of the craft.

From *Nature*, 9, 350, March 5, 1874.

international news

Protest about emigration from the Soviet Union

Vera Rich, London

THE right of the Jewish intelligentsia to emigrate from the Soviet Union to Israel has been raised once again by the fast, in Moscow, of David Azbel, a professor of chemistry, Vitalii Rubin, an eminent Sinologist, and Vladimir Galatskii, a talented but little known artist. In a statement issued on the first day of their protest, the three participants declared:

"We begin today a hunger strike, taking a step befitting political prisoners. We consider ourselves as such even though we live in Moscow in our flats and with our families. One can deprive a human being of his liberty even without arresting him. The case then, is of arrest without investigation, without a trial and without a date for release".

Since October 1971 when the Soviet restrictions of Jewish emigration to Israel were relaxed insofar as total numbers were concerned, it has become increasingly clear that the new policy was not to be applied on a basis of equality. According to a law of 1942, all persons wishing to emigrate from the Soviet Union are obliged to pay 400 roubles for an exit visa, plus 500 roubles for renouncing their Soviet citizenship—900 roubles in all (£450). In addition to this, however, a new principle was introduced on August 3, 1972—that all persons with higher education wishing to emigrate should first refund to the state the cost of that education.

In a press statement made through the Novosti agency (December 28, 1972) Deputy Minister of the Interior Boris T. Shumilin explained this policy in terms of economic pragmatism with a touch of socialist solidarity. "The Soviet Union", he said, "has no intention of acting as a philanthropist towards persons on whose education the state has spent considerable sums, or towards those capitalist states to which these persons are emigrating".

For this reason the repayment of fees is not demanded from those departing for socialist countries or those which have "taken the road to independent development", since special agreements exist between the Soviet Union and

these countries concerning the repayment of fees. Persons of retirement age and invalids are, he said, exempt from all repayments, and rebates are given in accordance with the length of time worked (for example, 75% rebate for a man with a working record of 25 years or a woman with a working record of 20 years).

One would expect that since the state demands such high 'transfer fees' for its valuable personnel (a record figure of 23,000 roubles paid by an unnamed Jewish composer was reported in February, 1973), it would use these human 'assets' in the best interests of the Soviet economy. This, however, is not the case. Although it is not illegal to apply for



David Azbel

emigration to Israel, clearly those who do so are dissatisfied with the Soviet regime. And dissatisfaction with the regime is viewed by the authorities as dissent and, in many cases, as mental illness.

Thus one has the anomaly that a scholar such as philologist Vitalii Shevuroshkin, refused an exit visa on the grounds that his services were "indispensable" to the Soviet Academy of Sciences, was shortly afterwards dismissed from his post. One presumes that no adequate replacement can be found!

Since March 1973, when the enforcement of this 'diploma tax' threatened to jeopardise Soviet-United States trade

agreements, it would seem that the repayment of higher education fees has no longer been enforced. But the journalist Victor Louis, who seems to have a special insight into the workings of the KGB, informed the Israeli newspaper *Yediot Aharonot*, that "although it had not been abolished, the tax would not in future be demanded". Inverting the proposition, this means that although for those intellectuals fortunate enough to be allowed to leave as a sop to opinion abroad (five left with much publicity at the time of the said trade talks), it remains unrevoked and could be reimposed at any time.

Instead of the diploma tax, recent policy has been one of ever increasing harassment of the would-be emigrant. He is seen as a dissenter and dismissal from his post is the least that he can expect. In the case of the electrochemist Dr Venyamin Levich, his own dismissal was followed by the exiling of his astrophysicist son to Siberia (in spite of suspected cancer of the stomach) and a curious attempt to impose the "academic oblivion" of Stalinist days.

The distribution of foreign scientific journals has been handled by the Institute of Information, which photocopies the journals and then distributes these copies to libraries and subscribers. This means that 'undesirable' material can be removed at this stage and, in order to cover up the omission, replaced by innocuous material such as book reviews from other issues.

In reproducing the *Journal of the Chemical Society*, No. 8, 1973, (which makes reference to Levich's work) the older blanking-out type of censorship was, however, adopted, so that references to the "Levich-Dogonadze" theory become references to the "... Dogonadze" theory throughout the Soviet version, except in one case where Levich's name remains by an apparent oversight (see Fig. 2).

Professor David Azbel, one of the three participants of the present 'hunger strike' was 61 years old when he applied, in May 1972, to emigrate to Israel and therefore, according to the Shumilin statement, should have qualified for emigration without payment of the diploma tax. His life history up to this time reads like a synopsis of some Solzhenitsyn novel. Orphaned at the age of nine—he was forced to watch his mother hanged during a White pogrom in the Civil War—he made his way as

A number of theories of oxidation-reduction and electrochemical electron exchange reactions have appeared. Common to several of these theories is a particular approximation method embodied in the use of the dielectric continuum representation of the electrically polarizable nature of the solvent system surrounding the reactants and products: the electron donor and acceptor species. In addition, one theory is fundamentally quantum mechanical; this theory has been developed to a considerable extent by Dogonadze and their associates.¹⁻⁴ The Dogonadze solution electron transfer theory¹ resembles the solid state polaron theory.⁵ Further, the Dogonadze theory owes much to the Born-Oppenheimer adiabatic separation of the transfer electron dynamics from environmental motions for its progress from the conceptual model, with its associated Hamiltonian operator, to the expression for the rate constant. The advantage in the application of the Born-

one of the numberless 'waifs of the Revolution' from his native Ukraine to Moscow, where distant relatives gave him a home. He graduated in 1935, just in time to be arrested in the first wave of Stalin purges. He was released in 1951 and was 'exiled' back to Ukraine, where he worked as an engineer until he was dismissed from his post in the wave of anti-Semitism at the time of the alleged "Doctors' Plot" against the life of Stalin.

After Stalin's death, he was 'rehabilitated', allowed to return to Moscow, where he completed his postgraduate studies, becoming a doctor of technical sciences in 1960 and a professor in 1961. In 1968 he became head of the Institute of Chemical Spirits and Organic Products in Moscow. During this time he published some 60 scientific papers, dealing with mass transfer, bubbling regimes and the kinetics of biochemical processes.

In May 1972, at the age of 61 (and therefore exempt from any 'diploma tax' according to Shumilin's pronouncements), he applied to emigrate with his wife and child to Israel. He was immediately dismissed from his post and the long harassment started. In December 1972, he was arrested with other Soviet Jews following a hunger strike outside the Central Telegraphic Office in Moscow and was sentenced to 15 days' imprisonment for "holliganism". In October 1973 he was reported arrested in Leningrad in a general round-up of leading Jews at the time of the Yom Kippur War. A few days later, he is said to have been in hiding in his Moscow flat, under constant surveillance by the KGB and afraid to leave, even to buy food, lest he be arrested. Although these last reports seem somewhat contradictory, the general impression of harassment is unmistakable.

So, with his friends Rubin and Galatskii, he now considers himself a "political prisoner", although as yet all three live in Moscow in their own flats and with their families. Compelled in 'the interests of the state' to remain in the Soviet Union at a time when it is ready to expel such 'undesirables' as Solzhenitsyn, they are undertaking his hunger strike to draw attention to the

anomalous position of the Soviet Jewish intelligentsia. Although the official propaganda sources stress that "tens of thousands" of Russian Jews are permitted to leave, these figures, say Azbel and his companions, only mask the 'human drama' of the Jewish intelligentsia, who "From the moment we applied for exit visas . . . have been victims of endless harassment, our dignity as human beings often degraded. We have had to face officials who could torture us at will. Our application to higher officials proving our just causes were answered by lies and mockery. Our protests were answered by repression".

New riot control agent for US Army

Colin Norman, Washington

A CONTROVERSIAL new riot control agent, which was developed in Britain, has been approved for use by the United States Army. Called CR, it is a highly potent irritant to the eyes and skin, and it is likely to be at least a partial replacement for CS, the riot control agent widely used in Vietnam. The first public indication of the approval of CR for use by US forces was contained in a letter sent last week by Dr Malcolm Currie, the director of research and development in the Department of Defense, to Wayne Owens, a Democratic Congressman from Utah.

Developed at Britain's Chemical Defense Establishment at Porton Down, CR (its chemical name is dibenzoxazepine), is considered by both the British and US governments to have some advantages over CS as a riot control agent, chief of which is that it can be sprayed rather than disseminated by a smoke bomb.

CS is relatively indiscriminate in its effects—when used in a wind, it tends to blow away from the target area, and even to blow back into the faces of the troops using it. There have also been reports that rioters can develop a tolerance for its irritant effects. CR, however, can be used as a liquid suspension in propylene glycol, which means that it can be sprayed directly at the target.

The agent was issued to British troops in November last year, amid considerable criticism because the British government had released only scant information about the toxicity of the agent. No further information has yet been issued in the United States, although the most comprehensive tests were carried out at the Edgewood Arsenal in Maryland. According to Currie's letter, the US government is precluded from releasing reports of those tests because the Department of Defense "is bound by the terms of the standardization agreements not to release information derived from the property of foreign governments". He added, however, that "the UK has now embarked on an active program to present information on CR in the scientific literature. We anticipate, therefore, early release of some technical publications concerning our investigations".

The most comprehensive data so far published on CR were contained in an article in an obscure British magazine, *Medicine, Science and the Law* (October, 1973), which was written by senior officials from Porton Down. The paper described tests on 150 male volunteers, and it described the effects of CR as being very sharp and painful, and they lasted for about 20 minutes. The agent also causes the victim to shut his eyes tightly.

As for toxicity, such information as there was in the article suggests that the toxicity of the agent is low, but it was entirely inadequate to gauge the long-term effects. The key data are contained in the reports of the Edgewood Arsenal studies, and until those are released, no adequate scientific judgement of the risks associated with exposure to CR can be made. It should be noted that the Himsworth Committee, which was set up by the British government to study the use of CS, strongly recommended that all new riot control agents should be thoroughly tested and the results opened up to public debate before they are issued to the armed forces. This did not happen with CR in Britain, and the United States government has quietly followed Britain's bad example.

Should pharmaceuticals be left alone?

Roger Woodham

WHAT precisely is the Labour Party's policy for the British pharmaceutical industry? The Association of the British Pharmaceutical Industry (ABPI), for one, is not waiting any longer to find out and has published a hard-hitting riposte to that part of the Labour Party Election Manifesto that deals with future

public ownership in the industry. Predictably the association's conclusion is "leave well alone".

The manifesto devotes all too little space to spelling out just what the party has in mind. A public holding, it says, is necessary in certain industries to allow control of prices, stimulation of investment, encouragement of exports . . . There are evidently several choices.

- Nationalisation of some or all British companies manufacturing pharmaceutical products (including the British ends of multinational companies in the extreme case) .

- The setting up of a state holding company which would buy a controlling share in specified companies.

- The floating of a new company in competition with the rest.

The permutations are almost endless. Perhaps the Labour Party has in mind simply hiving off to the state certain parts of companies (declining, for example, to involve the nation in the soft drinks or cosmetics markets). In any event the 'big six'—Glaxo, Beecham, Fisons, ICI, Wellcome and Boots—are likely to feel a cold wind if the public ownership policy is ever implemented.

The ABPI argues its case against public ownership by posing and answering 13 questions. Is there any evidence, the association asks for example, that nationalisation can benefit the health of the public and the national economy? Its answer is "no", and that nationalisation would "stifle research success and reduce the industry's £220 million plus a year export record . . ." Certainly the ABPI has a point about exports, for if foreign-based companies ceased to operate in Britain about half that export potential could be lost.

This is not such an unlikely state of affairs, for any government could, if it wanted, decimate the British pharmaceutical industry in its present form by taking over one company. (Glaxo, which has a turnover of about £200 million, is a possibility for it has the added attraction of having two factories in development areas, at Alzerston in Lancashire and at Montrose in Scotland.) Given the manufacturing base the government could then invoke clauses in the 1948 Patent Act and the 1968 National Health Service and Public Health Act, which allow it to abrogate drug patents for the benefit of the public sector. Few foreign companies would stay in Britain in these circumstances. And, of course, a government buying even a part share in a foreign-based company would simply be buying bricks and mortar and a disillusional staff; no new ideas would ever again reach it from its parent.

What of research? The ABPI document boasts that the pharmaceutical industry plans to double its present

research investment during the next few years and generally expresses approval for the way in which pharmaceutical companies set about things. The objection, however, is that it is hard for a private company to justify to itself research in areas that are not seen to be potentially profitable. Presumably a national pharmaceutical corporation, or whatever, would do much less screening of compound upon compound for signs of biological activity of a desired kind, and concentrate instead on identifying problem diseases and looking, more 'scientifically', for a cure.

There is talk among Labour politicians that the British pharmaceutical research budget according to Labour plans would make the present spending look like peanuts, but there are others who would hardly bother with research at all and would concentrate on manufacturing generic drugs extremely cheaply. The national drugs bill footed by the government might be reduced from 7% of the cost of the National Health Service to 3% or 4% in this way, hardly a handsome return for such an extreme move.

The right balance may well have been struck by Pfizer, whose research effort in Britain was mainly fundamental a few years ago but is now split almost equally between the fundamental and the pragmatic.

Making out a case for the Coalplex

John Wilson

THE National Coal Board (NCB) is asking the government to provide "about £40 million" for research and development as part of a £400 million investment programme. The NCB has already carried out a great deal of fundamental research into new ways of producing energy and raw materials from coal at the Coal Research Establishment, Stoke Orchard, Cheltenham. Now it wants to set up pilot plants to assess the feasibility of its ideas on a larger scale.

Describing the diversity of the work now in progress, Dr J. Gibson, Director of the Stoke Orchard establishment, says that it ranges from the production of carbon fibre to the use of colliery spoil in motorway construction. But he outlines a unifying theme behind much of the research—the creation of a 'Coalplex'. A Coalplex is a single site where several processes for the extraction of energy from coal, usually in the form of electricity or gas, can be linked to the manufacture of other coal products such as metallurgical coke. The processes may be combined in almost any permutation so a Coalplex could provide different coal products if the emphasis on

Kennedy floats a critical plan

Colin Norman, Washington

IN an effort to provide a highly visible channel for independent criticism of the Administration's biomedical research policies, Senator Edward M. Kennedy has proposed that a three-member Presidential panel should be set up to keep an eye on the programmes of the National Institutes of Health (NIH). The proposal stems from the fact that for the past two years, the Administration has favoured the National Cancer Institute and the National Heart and Lung Institute with large budget increases, chiefly at the expense of other areas of biomedical research. Kennedy, backed by a number of influential scientists, believes that this trend is creating considerable unbalance in the overall biomedical research effort.

The panel, which was proposed in a bill Kennedy introduced last week, would be akin to the President's

Cancer Panel, a body which now overseas the national cancer programme. It would consist of three members, one of whom would be the chairman of the cancer panel, and at least two of whom would be "distinguished scientists or physicians". Its brief would be to report directly to the President and Congress on any delays or blockages in the execution of NIH's programmes—a definition sufficiently broad to allow the panel to voice its opinion on most aspects of research policy.

The bill has been sent to Kennedy's Senate Health subcommittee, where it will probably be attached to another bill which proposes some relatively minor changes in the National Cancer Act. Since it is being cosponsored by Senator Jacob Javits, the ranking Republican on the health subcommittee, there is every chance that it will be speedily approved. Meanwhile, a similar bill is being introduced into the House of Representatives by Paul G. Rogers, the chairman of the subcommittee on health of the House Commerce Committee. Again, little opposition is expected there.

the various individual processes were altered.

One part of a Coalplex could be used to supply the raw material (such as 'lean' gas of low calorific value) for several different processes and the by-products of one process could be utilised by another. In this way the overall capital cost of a Coalplex would be reduced and its efficiency increased. Dr Gibson points out that with the old town gas system, only the quantity of the products—gas, tar and coke—could be altered, not their proportion. In a Coalplex, no unwanted materials need be made.

Dr Gibson emphasises, however, that the setting up of a Coalplex represents the culmination of the research effort at Stoke Orchard rather than the core of present plans. It is the building of pilot plants to test the individual processes that is most urgent now, he says.

Perhaps one of the most important of these processes is the fluidised bed combustion of coal (FBC). The coal is burned in a sea of coal ash given the properties of a liquid by the upward passage of air. Low grade coals may be used and indeed coal washings, composed of 50% water, 25% ash and only 25% combustible material, have been successfully burned. This raises the interesting possibility of recycling some coal tips.

The equipment required by an FBC system is cheaper and more reliable than conventional apparatus, says Dr Gibson, and the process is also less harmful to the environment. Being about 10% more efficient than existing generating stations powered by coal, any FBC station would require much less water for cooling. Moreover, by adding limestone to the bed of ash, the amount of sulphur dioxide given off can be substantially reduced. With the growing concern about thermal and atmospheric pollution, Dr Gibson feels that these are important points to consider.

Because FBC is an efficient method of releasing the energy in coal, it could form a base around which a Coalplex might be built. It can also be adapted to the direct gassification of the coal, thus providing a gas from which oil can be produced. But other processes are also needed to extract the various coal products and these, too, require the development of pilot plants.

One way of obtaining coal products is to dissolve the coal in a solvent such as anthracene oil. The solution can then be used in the manufacture of carbon fibre and electrode coke. Dr Gibson claims that this coke is of sufficient quality for use in the arc processes of the steel and aluminium industries. Another extraction process which Dr Gibson would like to see in pilot operation involves the vaporisation of the coal into compressed gases held just above their criti-

cal temperature. When the resulting mixture is drawn off and cooled the coal extract can be precipitated out.

In the long term processes such as these could be the initial steps in the preparation of liquid fuels and chemicals. These might then be processed using the techniques of the petrochemical industry. With its high content of hydrogen, the gas extract is particularly suitable.

The idea of a Coalplex was first proposed by Lord Zuckerman in 1971 and most of the fundamental principles have been known for some time (aviation fuel was made from coal in Germany during the Second World War). But why has the NCB been so slow to exploit this concept? The answer lies in the economics of the operation. Quite simply it was, until very recently, several times cheaper to produce most of these materials from oil. The NCB had been thinking of an economic Coalplex in terms of 10 or 15 years from now. Recent events in the Middle East and a growing shortage of carbon products have changed this outlook. That is why the board wants to take the expensive step out of the laboratory on to the pilot site.

Nevertheless, Dr Gibson is careful to point out that he envisages a situation where coal and oil are complementary to the needs of industry and not in competition. Oil is a natural resource which contains a lot of energy and it would be a shame, he says, if such a fine material were degraded to gas, carbon fibre and other more mundane uses. If coal were used to produce these basic materials, it would relieve oil of a considerable burden.

NASA and Europe look to the planets

Colin Norman, Washington

Discussions have been held between officials of the National Aeronautics and Space Administration and their counterparts in Europe on the possibility of a joint United States-European mission to Jupiter. During a recent visit to five European countries, NASA officials floated the idea of European cooperation with the United States on a project which would involve use of Pioneer-H, the redundant backup spacecraft for NASA's two Jupiter Pioneers. The possibility of a joint mission to the comet Eake in 1980 was also discussed. The European response was "very enthusiastic", and firm proposals are likely to be made by September.

Two chief choices are being discussed. The first would involve a launch in 1978 and Jupiter encounter in 1980 which would take the spacecraft on a

trajectory underneath the planet, to emerge north of the ecliptic plane. After Jupiter encounter, it would then go on towards the Sun, passing over the north solar pole and swinging round the Sun past the south pole. If the mission goes ahead, it would be the first spacecraft to be sent out of the ecliptic.

The second choice would be to launch Pioneer-H in 1980 on a Jupiter orbiter mission. Such a mission would extend the observations of the two Jupiter swingby missions.

Although the costs are not yet clear, the out-of-ecliptic flight would be the cheaper option. At present, Pioneer-H has a set of instruments which duplicates those carried on the Pioneer-10 and 11 swingby missions. An orbiter mission would require the development of retro rockets and some changes in instrumentation, while the Jupiter swingby, followed by the out-of-ecliptic solar flight would only require the addition of a few solar instruments.

The exact mode of cooperation is still being worked out, but as far as the orbiter is concerned, the idea would probably be that the Europeans would provide the retro rockets.

The cost of immunisation

Sally Owen

ABOUT 2,000 children suffering from vaccine damage in Britain are not eligible for compensation because of the terms of reference of the Royal Commission on Compensation and Civil Liability. After a recent report that permanent brain damage followed immunisation against whooping cough in one child in about 5,000, Mr Jack Ashley, a Labour Member of Parliament, has drawn attention to this anomaly in the law and has accused the government of "shattering complacency" in its attitude towards children damaged by vaccines.

A successful campaigner for compensation on behalf of Thalidomide children, Mr Ashley referred to a "veil of obscurity" shrouding the fate of thousands of children and he asked for urgent action to reduce the number of future tragedies. He has also proposed that compensation should be offered to those already severely damaged.

Following Mr Ashley's campaign in the House of Commons, an association formed last year in the interests of children damaged by vaccines has been receiving letters of support from parents.

The Association of Parents for Vaccine-damaged Children feels that Britain should have compensation schemes for the casualties of vaccine administration, as do most European countries. There

are no compulsory immunisation procedures in Britain, whereas they are common in the rest of Europe.

Compensation for damaged children was discussed almost a year ago at a conference held in Monte Carlo on vaccination against communicable diseases. Although there was a strong call for a code of guidelines for vaccine trials, it was pointed out that public concern would jeopardise future trials. Standards of conduct satisfying both scientific integrity and lay opinion would be required.

Another view expressed at the conference was that there is a good case for compulsory immunisation against some illnesses; for instance a child with quadriplegia due to poliomyelitis might be justified in suing his parents for negligence in not having had him immunised against the disease.

The compensation schemes practised in Denmark and Germany are controlled by the authorities, who are responsible for individual claims for damages. This means that if it is suspected that a vaccine is faulty the manufacturer can be sued.

The choice of vaccines used in European countries, including those for compulsory immunisation, differs widely and it is predicted that within the next few years closer agreement will be reached on the pattern of immunisation schedules.

In Britain concern is being expressed about the whooping cough vaccine which was first introduced nationally in 1957. Since then there has been a marked decline in the disease. It is, however, known that some children—there are no reliable figures—have been seriously damaged by the use of the vaccine. In 1972 general medical practitioners were given advice on possible adverse reactions to whooping cough vaccine and certain children were recommended not to receive it.

The Committee on Safety of Medicines was not entirely satisfied with the voluntary reports received on adverse reactions. The reports did, however, identify possible problems with adverse reactions in the various immunisation procedures. In fact neither the committee nor its predecessor, the Committee on Safety of Drugs received reports of direct adverse reactions to whooping cough vaccine.

Whooping cough vaccine is usually given as part of a triple injection for diphtheria, whooping cough and tetanus, or as a quadruple injection in which case poliomyelitis is the additional vaccine. Even though it is usually considered not possible to ascribe any adverse reactions to an individual vaccine the committee had received 503 reports of adverse reactions which included whoop-

ing cough vaccine; most reactions were however, minor.

In 1963 the number of notified cases of whooping cough started to increase again and have fluctuated about a number roughly one-fifth of the average in the decade before 1957. A survey carried out in 1969 on the whooping cough vaccines in use until 1968 suggested that although there was a marked decline in the disease, the vaccine had not been absolutely effective overall. The findings of the report suggested that in future the efficacy of the whooping cough vaccine should be kept under constant surveillance.

Since there are children definitely suffering from the effects of vaccines it would seem proper that the matter should be opened up and accurate figures revealed to substantiate the accusations now being made.

When Mr Michael Allison, Under Secretary for Health and Social Security, was questioned in Parliament on the success of immunisation he said that it could be measured by the virtual eradication of diphtheria and poliomyelitis. He also claimed that no immunisation procedure is entirely free from risk of ill-effects, even though he understood that about 170 reports of adverse reactions to vaccines of all types were received each year.

Mr Jack Ashley was not satisfied with Mr Allison's remarks and intends to submit a motion to Parliament calling for action on the cases of children harmed by administration of vaccines.

Prescribing safely for children

Fiona Selkirk

SHOULD potentially dangerous drugs be prescribed for children under the age of 10, in the hope that they may alleviate some relatively trivial complaint of childhood? This is a question often raised and highlighted once again by a recent report in the *British Medical Journal* (BMJ) (1, 261; 1974).

The report is the results of a seven and one-half year survey of the cases of accidental drug poisoning in children admitted to the Royal Hospital for Sick Children in Glasgow (carried out by Drs K. M. Goel and R. A. Shanks). Of the 60 children suffering from poisoning by one or other of two drugs (amitryptiline or imipramine), three-quarters were between the ages of two and four years, more than half were poisoned as a result of having taken an excess of the drugs originally prescribed for themselves or for brothers and sisters. Of these 60 children, 30 were admitted during the past 18 months, indicating that this situation is worsening. The remaining 43% of children suffering from the

effects of poisoning had swallowed the same drugs prescribed for adults in the family.

The drugs in question are tricyclic hydrochlorides, commonly prescribed as anti-depressants in adults, and have been found to have varying degrees of usefulness in the treatment of nocturnal enuresis (bed-wetting) in children.

There is no positive information about the toxicity of these drugs in abnormal quantities. There is no known antidote. Some people believe that serious manifestations invariably appear after ingestion but others believe that the severity of poisoning is related to individual tolerance and that the severity of poisoning should be assessed symptomatically.

Any case of accidental overdose of tricyclics in children is treated with close observation for 24 hours and the experience of the Glasgow group is that if the child survives that first critical period, it will probably recover completely, with no after effects. The symptoms presented are treated where possible. The heart is restarted mechanically; the lungs ventilated; convulsions treated with drugs and the contents of the stomach are always removed.

Opinions vary as to whether the use of these drugs has any lasting effect on the condition. Dr Shanks firmly believes that they do not. He feels that at best the effects are only transient and that it seems that as soon as treatment is stopped the situation reverts to the *status quo ante*. A general medical practitioner who has prescribed these drugs from time to time for his patients, and has found their effect "quite impressive", says the limiting factor to the duration of treatment is usually the patient. Either he comes back for a repeat prescription because "the tablets you gave him last time worked so well doctor" or he stops coming. The doctor admitted however, that he knew cases where the condition seemed to have cleared, treatment had stopped and then, perhaps six months later, the patient's mother was back in the surgery, asking for more, because the child had started being wet again.

This doctor does not believe in treating children of pre-school age but older children, who are likely to suffer socially from the effects of this condition, he believes should get whatever help he can give them. Parents should be given something positive to do, so that they feel they are being of some help to the child also.

Having been quite impressed by the effectiveness of these drugs in the treatment of enuresis in his patients, the doctor said that he was "pretty horrified" when he read of the effects that accidental poisoning seem to produce in

young children.

On the whole, doctors are at their own discretion as to what drugs to prescribe to which patients, and how much and how often the medicine should be administered. They have as guidelines the literature produced by the pharmaceutical companies marketing the drugs, reports in the medical and scientific literature, training courses at universities and colleges and, occasionally, directives from the Department of Health and Social Security, distributed through the local Executive Councils. There are many journals available free or on subscription—one such is produced by the Consumer Council—which give doctors information about medicines, contain advice on which drugs established for the treatment of one condition have a beneficial effect for a completely different disease. The pharmacologists then have to discover the reason for the link.

When doctors prescribe drugs, especially for children, they generally warn the patient of the dangers associated with the medicine and remind them of the necessity to keep all medicine out of reach of children. Dr Shanks says that when parents of children who had poisoned themselves with the drugs were told of the dangers, they were naturally very upset and many said that they had not realised just how dangerous they were. If the rationale for prescribing tricyclics for young children is that they will relieve the anxiety symptoms manifested by enuresis (and one accepts that there are occasions when their use is justified), then perhaps the warnings should be spelt out more fully.

There have always been claims for the effectiveness of some treatment for a particular disease or condition but, on the whole, the cures were not as dangerous as the illnesses. Today, perhaps, there is too much emphasis placed on a cure at all costs, or at least easing of symptoms, and the advances of the pharmaceutical industry, together with the pressures of society, are just a little too great on the doctor with the prescription pad and the distressed patient.

Congress to lose key nuclear advocates

Colin Norman, Washington

THE Joint Committee on Atomic Energy, which for years has held a tight rein on legislation dealing with all aspects of nuclear energy in the United States, is about to lose two of its most powerful, and certainly its most colourful, members. Last month, Craig Hosmer, the most senior House Republican on the committee, announced that he will be retiring from Congress at the end of the year. And last week, his comrade-

in-arms, Chet Holifield, announced that he, too, will quit national politics this year after a 32-year stint in Congress. Their departure is raising some speculation about the future for the joint committee.

Holifield, a crusty Democrat who was a charter member of the committee and its chairman in alternate Congresses between 1963 and 1971 (the chairmanship alternates between a Senator and a Congressman), has decided to step down rather than face a tough challenge in a primary election in his California constituency. In 1971, he became chairman of the House Committee on Government Operations, a move which put him in a strong position to intercept and reshape the Administration's proposals for government reorganisation, and his Joint Committee mantle then fell to Melvin Price, a Democrat from Illinois.

Hosmer, Price and Holifield between them have largely dominated the work of the joint committee since the late 1950s. They have devoted their time almost exclusively to the committee's work, often at the expense of other activities, and this has given them a depth of knowledge which their opponents find difficult to overcome. All three are strong advocates of nuclear power, and they have been constantly attacked by environmentalists in the past few years.

Thus, with two members of the troika about to depart, a leadership vacuum is likely to emerge in the committee. Whether that will diminish the committee's power on Capitol Hill remains to be seen, but other developments are likely to have a more important impact on its future role. In any case, the unique status of the committee makes it an extremely difficult body to get rid of, or even to change.

The committee derives much of its power from several factors. First, the Atomic Energy Commission has a legal responsibility to keep the committee "fully and currently informed" of its activities, a requirement which gives committee members complete access to the agency's plans and deliberations. Ralph Nader recently remarked in a hearing before the joint committee that a result of this requirement is that the committee "has acted more like a part of the executive branch than any other committee of Congress", and it is often accused of being a mouthpiece for, rather than a watchdog of, the Atomic Energy Commission.

Second, it is the only legislative committee of Congress which has responsibility for nuclear energy. Other matters are considered by separate committees in the House and the Senate, and interchange between committees is frequently important for bringing out an resolving important problems. According to the

joint committee's critics, the lack of interchange on nuclear energy matters often has the result that differences of opinion fail to surface in Congress.

And third, the committee was established by an Act of Congress, and thus its functions and structure cannot be altered unless Congress passes a bill to do so. Since such a bill would be referred to the Joint Committee, its future is entirely in its own hands.

One development could, however, have a profound impact on the committee. Congress is now considering a bill to create an Energy Research and Development Administration (ERDA), based on the laboratories of the Atomic Energy Commission, but including energy research and development programmes from other agencies. If Congress passes the bill, the AEC would be split in two—the regulatory functions would be put into a separate agency—and the joint committee would be left with authority over an agency with which many other committees would also be concerned. A bill to establish ERDA has already been approved by the House of Representatives, and is now under consideration by the Senate Government Operations Committee.

Whatever the prognosis for the ERDA bill, the joint committee will certainly be a different body without Hosmer and Holifield. Their hearing room style alone, which ranged from bitingly sarcastic to gentle bullying—but which was always incisive—will be especially missed from the committee's deliberation.

Hearing double

A CURIOUS piece of programming by the BBC on Monday February 11 produced "Scientifically Speaking" on Radio 3 commencing at 2125, and "Kaleidoscope" on Radio 4 commencing at 2130. As if this were not enough to strain credulity, listeners with two radios were then treated to a simultaneous discussion of black holes and X-ray sources, with special reference to Cygnus X-1, on each channel.

On Radio 4 Professor R. L. F. Boyd could be heard confounding the interviewer (who may have been intimidated by the knowledge that Professor Boyd is a member of the BBC's Science Consultative Group) with a moderately hairy description of the black hole phenomenon which made no concessions to the level of scientific interest of the "Kaleidoscope" audience; on the theoretically more highbrow Radio 3, the lucid discussion by Professors Martin Rees and Ken Pounds was, by contrast, so clear that confused "Kaleidoscope" listeners who switched channels hoping to catch the end of a concert might even have stayed for the end of the programme.

correspondence

Is anonymity necessary?

SIR,—As co-editor of a series of books (*Comprehensive Virology*, Plenum, 1974) I was recently taken to task concerning the advisability of anonymous reviewing of solicited chapters. We fully agree with this criticism and will in the future request our reviewers to submit their constructive critical comments over their signature, so that the author can ask for clarification or defend his position.

As a past or present member of the editorial boards of several journals as well as frequent reviewer and reviewee of papers, I would like to broaden this enquiry into the effect of anonymity in the domain of reviewing for scientific journals. I was recently asked to review, and advocated rejection of, a paper for a virological journal on the basis of factual comments which I would have been quite willing to sign. The editor sent me, out of courtesy, copies of his rejection letter together with the other reviewer's sarcastic poison-pen comments, also rejecting the paper. There was no justification for one civilised person insulting another in such manner (as well as a specified alternative "boring" journal that might be willing to publish such "routine and dull work"); that outburst was solely the joy of releasing adrenaline with anonymous impunity.

I have read many other reviews which were full of bias. In one recent instance the reviewer, not wishing to accept the well proven conclusion of the author, misquoted the literature to prove his point to an editor who would not have the time to verify his citations.

I have given this matter considerable thought. There is no question that much good comes out of careful and conscientious reviewing and that scientific communication would not be improved by abolishing it altogether. But anonymity tends to bring out the worst in people and it causes undesirable aspects of the reviewing system which I believe to be unnecessary. Thus I advocate that editorial boards could and should come out strongly in favour of reviewers identifying themselves when they detect factual errors, weaknesses in the argument, inadequate or erroneous citations, or lack of clarity in a manuscript. The author can then consider these comments in the same polite and considerate style that such reviews would show.

This suggestion does not preclude the reviewer expressing his conclusions di-

rectly to the editors if he feels this to be necessary or helpful. If he advocates rejection in his letter to the editor, and if this is supported by the factual arguments given to the authors which in turn are not easily and clearly countered by them, then the scientific level of the journal would not be diminished, and the state of mind of the scientific community would be noticeably improved.

Yours faithfully

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Protein Gap

SIR,—The Food and Agriculture Organisation of the United Nations and the World Health Organisation have just published jointly their latest report on energy and protein requirements of man based on a comprehensive review of 50 years research in the field. A comparison of the average requirements per person based on this report¹ with average intakes shows that there is no protein gap at the national level. In most countries the average protein intake exceeds the average requirement so considerably that inequality in the consumption also fails to explain the full extent of the incidence of protein malnutrition. Furthermore, in those few individuals whose protein intake is found to be inadequate, the deficiency of energy intake relative to requirement is even more marked. Clearly, what diets lack is not protein but energy to metabolise the protein people actually eat^{2,3} (Miller and Payne, 1969; Sukhatme, 1969). In the circumstances the United Nations call to intensify production of semi-conventional protein-rich foods for closing the so-called protein gap seems to have little to commend it to the developing countries (U.N. General Assembly Resolution 1970).

Surprisingly, however, the 1973 report incorporates a new interpretation of the meaning of protein requirement to help planners evaluate an apparently non-existent protein gap. This interpretation is that an individual eating below the recommended level, called a 'safe' level in the report (that is the average requirement plus twice the standard deviation), while not necessarily malnourished, runs the risk of developing protein deficiency and that the risk increases as the intake falls below the "safe" level.

The implication is that individuals should be counselled to eat at levels above the "safe" level and that countries should aim to increase their protein supplies to make this possible. Calculations based on the normal bivariate distribution model for intake and requirement, which is implicit in the new interpretation of requirement show that the needed increases are very large; so large in fact as to be considered "unrealistic of the situation in the developing countries"⁴. In effect this exercise implies that 97.5% of the population would have to eat at levels of overconsumption—or waste—several times greater than the average needs to limit the population at risk (that is below the "safe" level) to 2.5%. If it were not protein but some other nutrient, say Vitamin A, these levels would be in the toxic range!

Evidently the assumptions implicit in the new interpretation of requirement require careful evaluation. I have examined them and find that they are not supported by available data. In particular, I find that the basic assumption that the requirement of an individual, like his intake, is known and can be determined with an error of measurement which can be assumed to be negligible, is not borne out by the data. I find that intra-individual variability in requirement often accounts for a major part of the total variation. Consequently neglecting to take it into account gives a grossly exaggerated and misleading picture of the protein problem in the countries concerned. In a similar vein, such statements as "two-thirds of the world is hungry and malnourished" as was first suggested by Lord Boyd Orr and which continue to be repeated in evaluating the nutritional situation in the developing countries have their origin primarily in the failure to recognise the status of the variable quantified by requirement. Details will be found in Sukhatme^{5,6}.

Yours faithfully,

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¹ WHO, *Energy and Protein Requirements* 522, (Geneva, 1973).

² Miller, D. S., and Payne, P. R., *Proc. Nutr. Soc.*, 28, 225 (1969).

³ Sukhatme, P. V., *Indian J. med. Res. New Delhi*, 57, 2170 (1969).

⁴ Beaton, G. H., *Proc. Western Hemisphere Natl. Congress*, (Miami, 1972).

⁵ Sukhatme, P. V., *J. Roy. Stat. Soc. Series A* (in the press).

⁶ Sukhatme, P. V., *Proc. Nutr. Society*, 33, 36A (1974).

news and views

Have tachyons been observed?

ONCE upon a time discoveries in physics were made by experimentalists who used their skills to detect the new and the unexpected. Interpretation came later. Nowadays, however, it is as often as not the theorist who takes the initiative. An existing theoretical framework may be extended in a self-consistent way that leads to quite specific predictions concerning new effects. The experimentalist is then invited to devise an experiment that will 'verify' or 'confirm' the predictions. Elementary particle physics, in particular, illustrates this trend. The pion and the Ω^- particle are famous examples of successful predictions. Particles that remain on the 'wanted' list include the legendary quarks—particles of large mass and fractional charge—and the tachyons. The quarks, if they exist, would provide a neat interpretation of the regularities observed among the known elementary particles (SU3 symmetry). The tachyons would allow one to extend the ideas of relativity into the region where velocities greater than the velocity of light occur.

In the usual interpretation of relativity, it is stated that particle velocities v can never exceed the velocity of light c . The reason is that the total energy $m_0 c^2 [1 - (v/c)^2]^{-1/2}$ must be a real quantity. To obtain a real value for the energy when $v > c$ we must suppose that the particle concerned is one for which the rest mass parameter m_0 is not a real number. Instead we must put $m_0 = im$, with m real. If this extension to the theory is physically valid, then tachyons can exist and their total energy is given by $m_0 c^2 [(v/c)^2 - 1]^{-1/2}$. It follows that tachyon velocities are restricted to the range $v > c$ and that their energy diminishes as v increases. In other words, energy loss produces acceleration. Other, somewhat bizarre, properties can be expected. A charged tachyon, for instance, automatically fulfils the conditions for emission of Čerenkov light, even in free space. It must therefore radiate energy spontaneously and suffer continuous acceleration to higher and higher velocities. It has no possibility of 'uniform motion in a straight line'.

The experimental search for tachyons is based on the supposition that they can be produced during elementary particle interactions and can subsequently cause such interactions. Some investigators have performed bubble chamber plus accelerator experiments; others have made use of the cosmic-ray flux and have looked for tachyon production in the atmosphere. The bubble chamber studies apply the requirements of energy-momentum conservation to obtain the square of the mass of an unknown particle produced in the interaction. This is standard practice. For ordinary particles, the (mass)² is, of course, positive but for tachyons the value would be negative. So far, this search has been unsuccessful. The cosmic-ray search depends on the possibility of tachyons being produced in the early stages of development of extensive air showers. Since the tachyons travel (by definition) faster than c , they must reach the ground before the shower itself. If they are capable of producing a response in ordinary particle detectors, one should therefore observe particles associated in time with the air shower but occurring some tens of microseconds earlier.

An experiment of this kind is described by Clay and

Crouch in a communication on page 28 of this issue of *Nature*. With due caution, after a careful statistical analysis of their measurements, they conclude that they have indeed "observed non-random events preceding the arrival of an extensive air shower". This result should encourage others to continue the search. Until the measurements provide something more tangible in the way of estimated cross sections and other limitations on the parameters used to describe the particle, it is unlikely, however, that they will inspire more than polite interest among theoretical physicists.

From a Correspondent

Structure of transfer RNA

NUCLEIC acid research entered a new era, when a little more than a year ago, Kim, Rich and their colleagues at the Massachusetts Institute of Technology, reported their success in the X-ray analysis of a crystalline transfer RNA. Their studies, at successively 5.5Å and 4.0Å resolution (*Proc. natn. Acad. Sci. U.S.A.*, 69, 3746; 1972; and *Science*, 179, 285; 1973), enabled the overall shape of the molecule to be determined with a fair degree of certainty. They now report, on page 20 of this issue of *Nature*, their latest results at an increased resolution of 3Å, and their new electron density maps reveal considerably more detail than hitherto. Thus, whereas at 4Å, the ribose-phosphate groups were discernible as individual peaks, the higher resolution data have enabled these to be resolved into two separate peaks, in many instances with a consequent increase in the confidence of the electron density interpretation.

The dominant feature of the 4Å model of Kim *et al.*, in which the molecule has an overall L shape, has been amply confirmed in the new electron density maps. The course of the polynucleotide chain was followed by tracing the characteristic ribose-phosphate zig-zag which was clearly seen in many places, especially double-helical regions. Base pairs also can be easily observed in many regions, and the double-helical parts of the molecule are all well resolved, although one base cannot be distinguished from another. Difficulties were evidently encountered, however, when they tried to follow the course of the chain in the non-helical regions of the molecule—the so-called loop segments. These seemed to be due partly to chain irregularities, and also to ambiguities in the maps in these regions arising from extraneous peaks which the authors ascribe to bound cations. The two loops, pseudouridine and dihydrouracil, which are somewhat crowded together close to the corner of the L, suffer especially from these uncertainties. Likewise, the CpCpA acceptor stem of the tRNA molecule is not well defined, though this may be a consequence of the inherent flexibility and sloppy nature of this grouping.

Several other details that were not apparent in the earlier low resolution studies have emerged. Previously the molecule was seen to have the two arms of the L consisting of two roughly perpendicular double-helical branches, each of which consisted of two colinear stems. This picture has had to be modified so that now only one branch has colinearity, the other has the anticodon and the dihydrouracil stems mutually inclined at an angle which may be as much as 30°.

It is perhaps not too surprising, in view of their shortness, that none of the four helical stems has been found to be quite regular, and that they cannot be described in terms of exact helical parameters. The authors have also been able to distinguish various other interesting features of these stem regions; thus, purine bases at the end of the helices often lie in stacked arrangement with respect to them. Similarly, the G-U base pair which lies in the middle of the CpCpA stem is also stacked parallel to the other double-helical bases in this region. The anticodon loop of the tRNA molecule, which is involved in coding recognition during protein synthesis, is seen to have the bases on the outside of the loop, although, again, there are uncertainties here.

It is unfortunate that the features of tRNA that are of greatest functional interest are so far poorly resolved. The MIT workers are now extending their analysis to include all the reflections occurring in the diffraction patterns, which extend to a resolution of 2.3Å. It is to be hoped that this work, together with additional heavy-atom derivatives to improve the phasing, will result in a good definition of the complete molecular structure.

The next stage in tRNA crystallography—the structural analysis of species other than the yeast phenylalanine tRNA of Kim *et al.*—still seems some way off. Only then will one really be able to discuss the relations of tertiary structure to biological function by correlating structural features common between tRNAs. S.N.

Towards a more efficient use of cytotoxic agents

BIOLOGISTS who seek, by the introduction of defined chemical agents into the body of man to alleviate, remedy or cure its many ailments, have a peculiarly mixed credo. There are the "eye of newt and toe of frog" experts (*Macbeth*, Act IV, Scene 1) who are happy if they can produce a relatively effective agent which does not have any apparent and immediate side effects. Others, pursuing the notion of "sufficient unto the day is the evil thereof" (*St Matthew*, VI, 34) have devised agents of known toxicity which despite their side effects and lack of specificity are nevertheless used as the only ones available. In a situation where there are very few medicaments whose effects can be thoroughly understood, it is reasonable that the art of expediency should have been practised. On page 82 of this issue of *Nature* appears an article by Rubens and Dulbecco which is one of a series of attempts to provide a more rational basis for chemotherapy—in this instance of cancer.

Many cytotoxic agents have been used to attack cancer cells. In all instances there have been undesirable side effects on vital organs such as bone marrow so that the clinician has always had to consider the balance between desirable tumour cell eradication and potentially lethal toxicity. Attempts have been made to improve the specificity of action of these agents so that their cytotoxicity is concentrated in malignant cells. Ingeniously prepared 'latently active' agents which are themselves quite non-toxic but which can transform to toxic products selectively in tumour cells have been synthesised. Another approach has been to use larger molecular weight materials as carriers of the cytotoxic chemicals. Daunorubicin, for example, is more effective against the L1210 leukaemia in mice if it is administered as its intercalated complex with DNA (Trouet, Deprez-de-Campeneere and de Duve, *Nature new Biol.*, 239, 110; 1972). The complex seems to be taken up selectively by tumour cell endocytosis. Inside the cell the DNA of the complex is digested by lysosomal enzymes and the daunorubicin is released in its active form and attacks nuclear DNA.

The results in the laboratory have been of sufficient interest to lead to a clinical trial of the complex in the treatment of myeloid leukaemia (Sokal, Trouet, Michaux and Cornu, *Eur. J. Cancer*, 9, 391; 1973). Proteins have also been used as carriers of anti-cancer agents and albumin has been shown to be selectively incorporated into tumour areas (Busch and Greene, *Yale J. Biol. Med.*, 27, 339; 1955). Similarly, fibrinogen seems to concentrate in the necrotic regions of tumours (Islaker, Cerottini, Jaton and Magnenat, *Chemotherapy of Cancer*, edit by Plattner, Elsevier, Amsterdam, 1964) and attempts have been made locally to irradiate cancers by the use of radioactive iodinated fibrinogen and anti-fibrin antibody. Globulins, fibrinogens and albumins have been covalently bound to alkylating agents and antimetabolites in a further attempt to concentrate the cytotoxic agent in tumour cells (Wade, Whisson, and Szekerke, *Nature*, 215, 1303; 1967; Mathe, BaLoc and Barnard, *C. r. hebdom. Séanc. Acad. Sci., Paris*, 246, 1626; 1958; Larsen, *Eur. J. Cancer*, 3, 163; 1967). In some instances covalent attachment to protein has increased the selectivity of action of the anti-tumour agent. One interesting finding was that nitrogen mustards merely absorbed (and not covalently bound) to protein could also be more selective than the mustard alone (Wade *et al.*, *loc. cit.*).

An extension of this work and one more likely to give greater specificity has been to attach cytotoxic agents to tumour specific antibodies. Alkylating agents conjugated to antibody directed against tumour cell antigens have been quite effective (Ghose, Cerini, Carter and Nairn, *Brit. Med. J.*, 1, 90; 1967), but more surprising has been the finding that the alkylating agent chlorambucil merely absorbed to an immunoglobulin fraction is a much better inhibitor of an experimental tumour than either chlorambucil alone or absorbed to a normal globulin fraction (Ghose, Norvell, Guclu, Cameron, Bodurtha and MacDonald, *Brit. Med. J.*, 3, 495; 1972). This is surprising since the absorbed alkylating agent would be expected to dissociate from the globulins on injection. The basis for the improved selectivity is thus not at all clear, but the results have been confirmed by the related work of Flechner (*Eur. J. Cancer*, 9, 741; 1973) who showed that anti-Ehrlich ascites globulin complexed with chlorambucil could cure mice bearing the Ehrlich ascites tumour.

In an attempt to explain this phenomenon, Rubens and Dulbecco (this issue) have shown that chlorambucil and a tumour specific globulin are indeed synergistic but that there is no requirement for physical absorption between the two since separate addition of the two components can still bring about the desired effect.

Obviously the cytotoxic agent and the tumour specific globulin act together, but not by the protein acting as a carrier. The mechanism once elucidated may lead to more efficient use of the cytotoxic agents in the treatment of cancer.

T.C.
A.D.

C-type viruses of primates

BIOLOGISTS who hanker after new species of organisms may profitably examine primates for hitherto undiscovered viruses. In recent years more than twenty new herpesviruses have been described, and now several groups under contract with the Virus Cancer Program of the US National Cancer Institute have detected RNA tumour viruses in normal or malignant tissues of primates.

The first virus, known as Mason-Pfizer virus, was isolated by Chopra and Mason (*Cancer Res.*, 30, 2081; 1970) from a mammary tumour of a Rhesus monkey. The Mason-Pfizer virus has a morphology intermediate between the B-type

(mammary tumour viruses) and C-type (leukaemia-sarcoma viruses) characteristic of mice and other vertebrates. Then a C-type virus was isolated from a sarcoma of a woolly monkey by the comparative oncology group at the University of California at Davis (Theilen *et al.*, *J. natn. Cancer Inst.*, **47**, 881; 1971) and this virus was found to induce sarcomas when inoculated into marmosets (Wolfe *et al.*, *J. natn. Cancer Inst.*, **47**, 1115; 1971).

The Davis researchers also isolated a C-type virus from a lymphosarcoma of an ape, the white-handed gibbon (Kawakami *et al.*, *Nature new Biol.*, **235**, 170; 1972). Several lymphomas and leukaemias of gibbons have been reported lately, and C-type particles have also been observed in the only other one examined (Snyder *et al.*, *J. natn. Cancer Inst.*, **51**, 89; 1973). These observations, however, apply to gibbons which have been kept in captivity for several years and some have been subjected to experimental procedures such as X irradiation which may have rendered those animals susceptible to unusual infections. Immunological and nucleic acid hybridisation studies indicate that the viruses isolated from the woolly monkey and the gibbon are very closely related. Considering how widely separated in evolution and geographical distribution are the New World monkey and the ape, it seems possible that at least one of the C-type viruses has been acquired in captivity from another source, rather than that they represent natural infections for both species.

Three recent reports describe C-type virus particles associated with primate placental tissues. Schidlovsky and Ahmed of Pfizer Inc. (*J. natn. Cancer Inst.*, **51**, 225; 1973) observed particles by electron microscopy budding from the syncytial trophoblast in each of four Rhesus monkey placentae and also in erythroblasts of foetal haemopoietic tissues. Kalter and his colleagues at the Southwest Foundation for Research and Education, Texas (*Science*, **179**, 1332; 1973) independently observed C-type particles associated with the placenta of baboons. The virus particles were prevalent at the syncytial trophoblast of each of thirteen normal baboon placentae at various stages of gestation. These findings naturally led Kalter and his colleagues to investigate human tissue (*J. natn. Cancer Inst.*, **50**, 1081; 1973) in which they also claim to see C-type particles, though very few in number, in four of six human placentae. Each placenta required several hours of screening with the electron microscope before particles plausible enough for publication were photographed.

Kalter generously provided a baboon placenta to Todaro's laboratory at the

US National Cancer Institute and the isolation in infectious C-type virus is reported in this issue of *Nature* (page 17). The placental tissue was cocultivated with various mammalian cell lines and virus was found to replicate most efficiently in a canine thymus cell line. Molecular hybridisation studies using complementary DNA transcribed from the RNA of the propagated virus do not indicate any homology between the baboon virus and other C-type viruses, including those isolated from the woolly monkey and gibbon. There is, however, substantial homology with DNA extracted from normal baboon placenta and liver and with RNA extracted from the placenta.

These results suggest that the C-type virus represents an endogenous, vertically-transmitted virus of baboons, that is normally expressed in the syncytial trophoblast but not in most other tissues. No doubt several laboratories are now busy cocultivating human placental tissue with every mammalian cell line that comes to hand in the hope of propagating an endogenous human C-type virus. They may well be successful.

From a Correspondent

Replication in synchronised cells

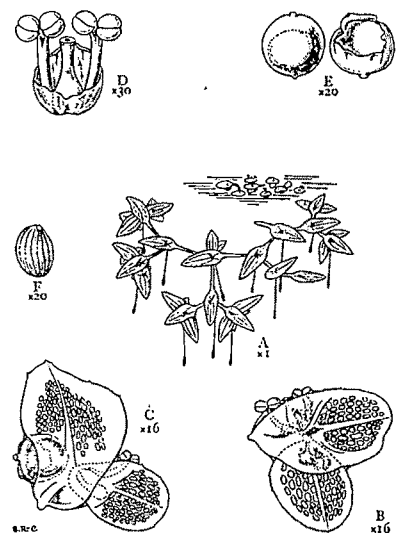
from a Correspondent

Non-random DNA replication during S phase of cultivated cells has been known since the early days of autoradiography. Moreover, analysis of the replication time of easily purified specific DNA sequences, such as the nuclear satellite DNAs found in many species of animals and plants, suggests that the ordering of replication is regulated by the DNA sequences involved. Although the exact point of replication of a particular DNA sequence may depend on the physiological state of the cell—for instance normally mouse satellite DNA replicates late in S phase but it seems to replicate in early S phase in cells infected with polyoma virus (Smith, *J. molec. Biol.*, **47**, 101; 1970)—it seems to be constant from one cell generation to the next.

Several descriptions exist for the replication time of the multiple genes for ribosomal RNA but they may be criticised on two grounds; one is lack of sensitivity of the technique used for assaying rDNA and the other is the inadequacy of the technique used for synchronising the cells. In a recent issue of the *Journal of Molecular Biology* (**82**, 303; 1974) Stambrook reinvestigates the temporal replication of ribosomal genes. He used Chinese hamster ovary cells synchronised by the 'wash off' procedure for collecting cells in meta-

Last of the line

THE publication of part XXXI of Stella Ross-Craig's *Drawings of British Plants* (Bell, 1973) brings to an end a series which started life in 1948 with Ranunculaceae and which has covered all British flowering plants except the Cyperaceae and the Graminae which have been illustrated adequately elsewhere. Lemnaceae (duckweed), Alismataceae (water plantain), Juncaginaceae (arrow grass), Potamogetonaceae (pondweed) and Zosteraceae (grass wrack) are among the families included in the last part. This figure shows the form of the ivy-leaved duckweed *Lemna trisulca*.



phase together with a highly sensitive assay for newly synthesised rDNA. Synchronised cells were labelled with bromodeoxyuridine (BUdR) for successive intervals of 2 h covering the 8 h duration of S phase and the DNA was isolated. Newly replicated DNA will be labelled with BUdR in one strand and will band in a neutral CsCl gradient at a greater density than the unreplicated DNA. Following banding in CsCl gradients, DNA fractions were denatured, loaded on to filters and challenged with a very high specific activity complementary RNA made *in vitro* using purified *Xenopus* rDNA and *Escherichia coli* RNA polymerase. The proportion of rDNA replicating during the BUdR pulse is equal to the proportion of cRNA hybridising to high density DNA, and the proportion of total DNA replicating during the same 2 h is given by the proportion of total optical density banding at high density. Although the nucleic acid hybridisation reaction is heterologous, Stambrook found that it has high fidelity. The cRNA is competed to a high level with Chinese hamster rRNA and also hybridises to a high density

component of total DNA in the same position as authentic ^3H -labelled Chinese hamster RNA.

Stambrook found that approximately 50% of rDNA replicates during the first 2 h of S phase during which time only 27% of the bulk DNA replicates; most of the remaining rDNA, together with 36% of the bulk DNA, replicates during the next 2 h. Studies with other mammalian cell systems have given very different results, for instance HeLa cells replicated ribosomal genes during the whole of S phase (Balazs and Schildkraut, *J. molec. Biol.*, **57**, 153; 1971) and rat kangaroo cells replicated them at the end of S phase (Giacanami and Finbel, *J. molec. Biol.*, **70**, 725; 1972). It is possible that the lack of a discrete replication time in HeLa cells results from a breakdown of some regulatory mechanism which coordinates the replication of rDNA and which is responsible for the apparent synchrony of replication of rDNA in other cells. A more likely explanation, since the multiple ribosomal genes in HeLa cells are localised on five separate chromosomes whereas those of Chinese hamster and rat kangaroo are probably located on one chromosome, may be that coordination of replication is related to locus, separate loci being under independent control. Although against this interpretation is the observation that satellite DNAs are spread over several chromosomes and replicate together at the end of S phase.

One criticism of Stambrook's experiments is that there is no indication of the molecular weight of DNA used in the CsCl gradients. It could be argued, since ribosomal RNA coding sequences are known to be intermingled with spacer DNAs of approximately equal length, that early replication of spacer DNA in the absence of replication of ribosomal RNA coding sequence would lead to the latter banding at higher density. Banding of DNAs with molecular weights less than one complete rDNA repeat unit (8×10^6 daltons) should in principle distinguish this possibility.

Stimulation of tumour cells by antibody

from a Correspondent

THE ways and means by which the immune system elects the destruction of tumour cells have always held centre stage in tumour immunology, but a gentle push towards the wings now seems to be in the making. That push is being exerted by immunologists who maintain that the immune response, in addition to its ability to inhibit tumour growth, can—in the appropriate conditions—

stimulate it. This immunostimulation theory was first put forward by Prehn a few years ago, the essence of it being that a weak immune response could encourage growth of nascent tumours, whereas a more potent immune response could effect the opposite result.

How this effect might occur is the subject of a paper by Shearer, Philpott and Parker in a recent issue of *Science* (**182**, 1357; 1973); the authors studied the effect of adding specific antisera to various cultures of human or mouse tumour cell lines. In all cases it was found that the addition of small concentrations of antibody brought about enhanced cell growth, as assessed by increased cellular incorporation of radioactive precursors of nucleic acids. Conversely, high concentrations of antibody inhibited cell growth, effects which clearly mimic Prehn's earlier *in vivo* studies on the effect of transferring high or low numbers of immune lymphocytes along with tumour cells into a non-immune host (*Science*, N.Y., **176**, 170; 1972). Furthermore, the authors found that the effect could be duplicated by coupling a chemical haptenic group to the tumour cells and subsequently adding various amounts of antibody with specificity for the chemical grouping to the cell cultures.

These results, taken together with Prehn's, clearly indicate that antibody molecules against tumour cell surface components can induce alterations in growth behaviour which are strikingly similar to those seen when lymphocytes are used as target cells, and imply yet another mechanism by which antibodies can enhance tumour growth.

An afterthought of these experiments is whether antibody of irrelevant specificity, if it can be made to interact with a tumour cell, will induce a similar kind of stimulatory effect. Such a union is made plausible by the surprising demonstration that a variety of non-lymphoid tumours in the human have receptors for antigen-antibody complexes (so-called Fc receptors) which recognise the Fc region of immunoglobulin molecules when complexed to specific antigen (Tønder and Thunold, *Scand. J. Immunol.*, **2**, 207; 1973). Such receptors have been described as an array of cell types including macrophages, mast cells, granulocytes, neutrophils and lymphocytes. Furthermore, it is well known that immune complexes can stimulate cell division of lymphocytes *in vitro*. Considering the similarity of certain populations of tumour cells and lymphocytes with regard to the effects that specific antibody on their growth characteristics, as well as the presence of Fc receptors, it might not be too surprising to find that certain kinds of immune complexes can stimulate or inhibit tumour cell growth.

Do mantle plumes produce copper?

from our Geomagnetism Correspondent

ALTHOUGH the various hypotheses making up the new global tectonics have combined to form a viable framework for the behaviour of the Earth as a whole, much still needs to be done in interpreting more local geological data in terms of the wider, and hopefully unifying, concepts. Indeed, the job of interpreting new data and reinterpreting the old is likely to be the main preoccupation of Earth scientists for many decades to come, not only in connection with 'pure' geological phenomena but also as a first step towards more successful methods of prospecting for mineral resources. But there are dangers, for in spite of the success of the new global tectonics, some of the basic concepts involved are still matters of dispute; and a too premature and uncritical application of them in more localised situations could easily lead geology, and especially economic geology, into a series of costly blind alleys.

The problem is well illustrated by Livingston's attempt (*Earth planet. Sci. Lett.*, **20**, 171; 1973) to reinterpret the formation of porphyry copper deposits in the south-western United States in terms of the activity of a mantle plume. The deposits in question are "geochemically distinctive petrological entities" containing anomalously high concentrations of copper, sulphur, molybdenum, gold, silver and rhenium, and are related genetically to the intrusive phases of intermediate to silicic volcanic complexes. Potassium-argon dating, for example, has shown that the igneous intrusion and the metallisation were very close in time. On the other hand, there are both barren and productive intrusions with similar lithophilic element compositions, which leads Livingston to suggest that the formation of those intrusives carrying metallic sulphides of commercial significance is governed by some "unique factor". This unique factor, he then claims, is a mantle hot spot, although this does not necessarily mean that the sulphur and metals are derived directly from the deep mantle.

The chief evidence in favour of metal formation by hot spot comes from the distribution of the deposits in space and time. All of the porphyry deposits considered by Livingston have potassium-argon ages in the range 52–72 million years, which is identical to the range of ages for the associated volcanic and intrusive rocks and coincident with the time span of the classical Laramide orogeny. But when the ages of the individual deposits are

examined in greater detail, they are not found to be distributed completely randomly within the geographical area concerned. Although there is considerable overlap there is a definite trend of decreasing age to the south-east (actually S40°E), a pattern which Livingston concludes could not be due to slow cooling. Instead, it appears that the "source" of the deposits moved towards the south-east at a rate of $3.57 \pm 0.65 \text{ cm yr}^{-1}$ —a rate sufficiently typical of plate motions to suggest that such motions are responsible for it.

The first plate tectonic feature to come to mind in this connection is the postulated subduction zone off the Pacific coast of Baja California, but although this is of the right age it would be unlikely to produce deposits whose ages vary along a line parallel to it. The alternative is a magma source which either moves towards the south-east in the lithosphere or asthenosphere, or remains stationary in the asthenosphere or mesosphere as North America moves north-westerly above it in response to global tectonic forces. The latter, adopted by Livingston as the simplest hypothesis, is a mantle plume which, taking account of the areal spread of the copper deposits, had an apparent surface diameter of 450 km. As far as the existence of copper emplacements is concerned, it also had a rather limited life of about $20 \times 10^6 \text{ yr}$, although the discovery of further evidence extending its existence cannot yet be ruled out.

On the wider scale, Livingston admits that the direction of plate motion implied by his hot spot appears to be inconsistent with the motions postulated by Dietz and Holden (*J. geophys. Res.*, **75**, 4939; 1970) and Phillips and Forsyth (*Bull. geol. Soc. Am.*, **83**, 1579; 1972) but suggests that his data represent motional details not resolved by "the more generalised approach". More critically perhaps, there is an apparent inconsistency with the directions of North American plate motion postulated by McGregor and Krause (*J. geophys. Res.*, **77**, 2526; 1972) and others. McGregor and Krause, for instance, assuming the Corner Seamounts of the North Atlantic to be the result of a hot spot, concluded that part of the North Atlantic moved in the S65°W direction subsequent to $74 \times 10^6 \text{ yr}$ ago. In fact, Livingston is able to show that this motion and his data are consistent if during the relevant period the North American plate rotated in a clockwise direction at an average rate of 0.35° per 10^6 yr about a pole located at about 50°N, 64°W, although to the extent that these figures are based on only two supposed plume traces they are probably not too accurate. Finally, Livingston claims that

his hypothesis is consistent with the available palaeomagnetic and palaeoclimatological data.

It is all so plausible; yet at the same time it somehow lacks conviction. Livingston is certainly right to document the degree to which the relevant data are consistent with the hot spot hypothesis, but for the time being it should be remembered that the very existence of hot spots and mantle plumes is widely disputed. The hot spot/mantle plume, though the least widely accepted of the phenomena associated with the new global tectonics, may ultimately become a part of the geological consensus. In the meantime, however, the dangers of introducing more and more of them to account for more and more localised phenomena should not be overlooked.

Attaching to the membrane

from our
Molecular Genetics Correspondent

ANY idea that the chromosome of *Escherichia coli* might possess only an indistinct structure was dispelled by the report of Stonington and Pettijohn (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 6; 1971) that it can be recovered from lysed cells as a compact structure sedimenting very rapidly, and further rebutted by Worcel and Burgi (*J. molec. Biol.*, **71**, 127; 1972) who found that this structure is organised in a particular manner. Worcel and Burgi suggested a model in which the *E. coli* chromosome is highly folded, with from 12–80 loops each maintaining a supercoiled structure; the loops may be attached to a core of RNA. Variations in the size of the folded chromosome (from 1,300–2,200S) were attributed to differences in the amount of DNA present, this depending on its state of replication. In an extension of this work, Worcel and Burgi now turn their attention to the membrane attachment of the folded chromosome and its relationship with the replication of DNA in the cell cycle (*J. molec. Biol.*, **82**, 91; 1974).

Two types of folded chromosome particle can be obtained from *E. coli* cells depending on the conditions of lysis. At low (0–4°C) temperature, the released particles sediment at 3,000–4,000S, whereas at room temperature the folded chromosomes sediment at 1,300–2,200S. At intermediate temperatures, both types of particle are found in the same lysate and can so be directly compared. Examining these two types of particle, Worcel and Burgi found that the protein:DNA ratio is, always some ten times greater in the heavier class; the amount of nascent RNA (corre-

sponding to the entire cellular content) remains the same. The dense particles appear to represent chromosomes attached to the membrane, whereas the lighter ones correspond to chromosomes not so attached. (Worcel and Burgi suggest the nomenclature "membrane-attached chromosomes" for the heavy preparation and "folded-chromosomes" for the lighter preparation.) Folded chromosomes can be released by solubilising the phospholipids of the membrane-attached chromosomes. Core RNA polymerase is the main protein in the released folded chromosomes, but the membrane-attached chromosomes have in addition a large content of membrane proteins.

Membrane-attached chromosomes cannot be obtained from cells which have been starved for amino acids (this treatment allows completion of current rounds of replication but prevents initiation of new rounds); all of the DNA from these cells sediments as the light form of the folded chromosome. But when leucine is added to the starved cells, the chromosomes appear to re-attach to the membrane. Although there is a lag of 30 min before replication recommences after the addition of leucine, all the chromosomes become membrane-attached within 20 min. And when amino acid starvation is imposed during replication, chromosomes continuing DNA synthesis appear to remain attached to the membrane. Association of the folded chromosome with the membrane thus seems to be directly related to DNA replication.

Earlier experiments have, of course, suggested that the origin and replicating forks of bacterial DNA are enriched in membrane fractions. But there are technical difficulties which make qualifications necessary in the interpretation of almost all of these experiments. To provide a critical test of the hypothesis originally proposed by Brenner, Jacob and Cuzin in 1963 (*Cold Spring Harb. Symp. quant. Biol.*, **28**, 329), that the replicating chromosome of *E. coli* is attached to a site on the cell membrane and that attachment to this site could be the critical event which controls cycles of replication, therefore requires, as Worcel and Burgi point out, experiments utilising procedures which do not alter the properties of the membrane-DNA complex as the cell is lysed. Just such criteria seem to be met in these latest experiments.

The reason for the increased sedimentation velocity of the membrane-attached form of the chromosome is not known, but may be due to either or both of the extra mass of the membrane fragments attached to the DNA or its mode of folding. In a second article, Delius and Worcel report electron-micro-

croscopy of the two types of chromosome preparation (*J. molec. Biol.*, **82**, 107; 1974). Both exhibit highly folded and tightly supercoiled DNA. The membrane-associated chromosomes show DNA fibres attached to one or two membrane patches and, since no free ends can be seen, the DNA is presumably intact in its usual circular state. The folded chromosomes seem to be less stable, more frequently generating breaks in DNA during preparation.

The topological problems posed by this compact structure of the chromosome remain formidable. Since strands of RNA can be seen in the folded structures, transcription must take place from sequences of DNA within the particle. Similarly, replication seems to proceed while the chromosome retains its compact structure. How DNA is able to unwind for these processes and how two daughter chromosomes may separate are among the most intriguing of the topological questions as indeed are problems concerning the access of enzymes and regulator proteins to particular sequences of DNA within the chromosome structure.

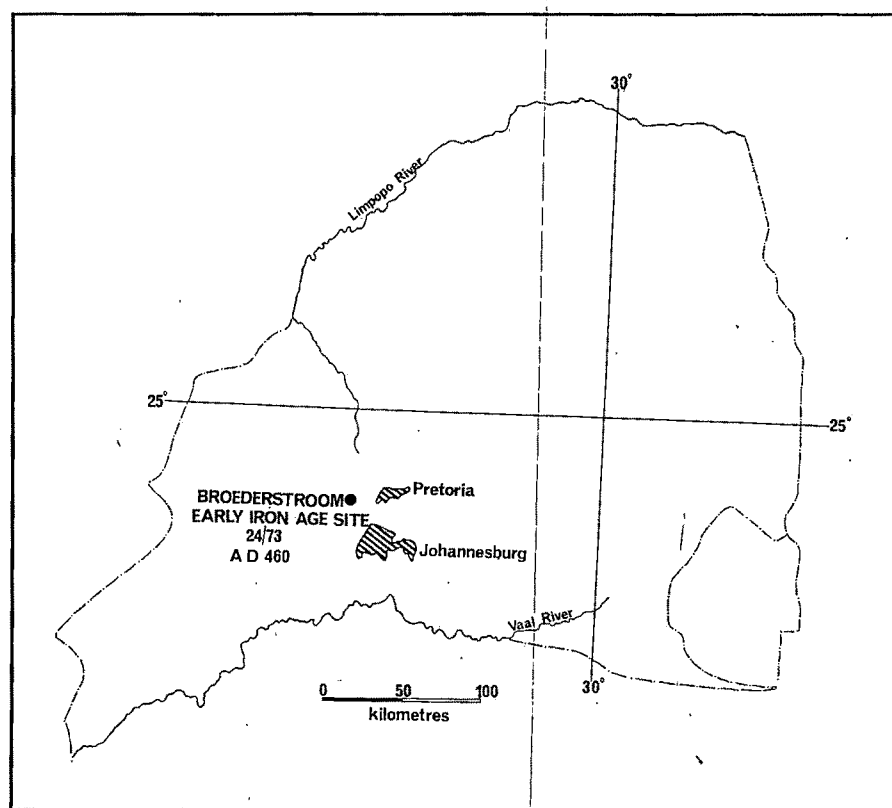
Early evidence of South African negroid

from a Correspondent

THE outline of an Iron Age village, which may represent the earliest and most intact village yet found in Africa south of the Sahara, has been discovered at Broederstroom near the Hartebeespoort Dam in Transvaal by a team led by Professor Revil Mason of the University of the Witwatersrand. The excavations followed up initial reports by Dr A. van Genderen who found the site. The material uncovered includes the earliest evidence of the South African negroid yet found and possibly also of domestic cattle.

The excavations at the site (no. 24/73) were conducted during the summer of 1973 in the grounds of the Leiden Observatory on the south bank of the dam and exposed thirteen huts and two iron slag floors on the perimeter and interior of an ellipse enclosing approximately 15 acres. No trace of stone walls was found suggesting that the village was enclosed by stockade. Among other material recovered were many thousands of sherds, a copper chain, disk ostrich shell beads, a cowrie shell, many hundreds of sandstone artefacts with 8-10 mm grooves (apparently for making shell beads), a few grindstones and some human remains.

Mason believes that the pottery was influenced by first millennium AD Iron Age pot makers in Zambia and in Malawi at Nkope and even as far north



as Kwale in Kenya, and that it is also related to pots made in the coastal regions of Natal and as far south as Pondoland, where pottery similar to that in the 24/73 assemblage was found in the 1930s by J. F. Schofield. The single cowrie shell also suggests contact with the coast. The Broederstroom pottery form includes pots with everted rims, bowls with carinated profiles and curiously thickened rims, lugs and one spout. Decoration is broadline incision of triangular punctate only; stamping is absent.

The human remains suggest links with first millennium burial ritual at sites in northern Tanzania reported by Odner (*Azania*, **6**, 89; 1971.) Part of a maxilla of an adult human lying on the base of a clay pot next to part of a mandible of a 6-12 year old child, and parts of a human leg skeleton were recovered. These remains, according to Professor J. van Reenen of the University of the Witwatersrand School of Dentistry, fall within the range of present-day South African negroids, and, with a radiocarbon date of AD 460 \pm 50 (obtained by R. Protsch at the University of California, Los Angeles), they are the earliest evidence of the South African negroid yet found.

Sheep or goat mandibles and molars of domestic cattle were also recovered and were identified by R. Welbourne. The cattle molars may be the earliest trace of domestic cattle yet found in South Africa, but their stratigraphy requires confirmation by the excavations planned for 1974. No plants were found during 1973, but it is possible

that flotation methods may reveal them during the next season.

Mason is confident that site 24/73 represents the earliest relatively complete settlement known to date in southern Africa, and that the pottery and burial methods indicate links with first millennium AD Iron Age cultures as far north as Kenya. The size of the slag floors, approximately 8 x 4 m each, and the quantity of grooved stones for bead making, suggest production in excess of local needs and may indicate trade links with neighbouring settlements not yet discovered. Mason has found similar pottery in Kruger Cave at Olifantsnek approximately 58 km west of the new site, and also near Thabazimbi 140 km north of 24/73. These records, together with those of other scattered Iron Age sites in Transvaal and Swaziland (see Mason, *Curr. Anthropol.*, **14**, 485; 1973), suggest that Iron Age settlement of South Africa during the first millennium AD was widespread. With the evidence of Broederstroom 24/73, archaeologists will now be able to relate these records to a village organisation of a kind associated with present-day Bantu speaking people in the rural regions of southern Africa.

How oil spills may affect the tundra

from our Plant Ecology Correspondent

THE exploitation of the oil fields in arctic North America has led to an increasing demand for information relat-

ing to its possible effects on the flora and fauna of the tundra biome. It is feared that the peculiar structure of tundra soils may render the system more sensitive to disturbance than has proved to be the case in more temperate climes. Below a depth of between 35 and 60 cm the soil is permanently frozen; the 'active layer' above this thaws and freezes according to the season. The application of physical pressures in the form of heavy vehicles is one form of disturbance which could upset this delicate system. Bellamy, Radforth and Radforth (*Nature*, 231, 429; 1971) found that vegetation in wet areas regenerated well following compaction—ruts disappeared within 5 years—but ruts in dry areas were still distinct even after 20 years.

Damage to surface vegetation in tundra could have more than aesthetic significance; erosion of the superficial layers of peat could have economic repercussions if vehicular transport became difficult and communications were affected.

Attention has now turned to the outcome of oil spills in such terrain, for in time such an event must be inevitable. Not only is it liable to be toxic to the surface vegetation, but it could also influence the radiation balance of the system with possible effects on the permafrost. Wein and Bliss (*J. appl. Ecol.*, 10, 671; 1973) have now described the outcome of experimental spills of crude oil in the Mackenzie Delta area of north-west Canada, 115 km from the Arctic coast. They chose different plant community types for their experiments, ranging from black spruce-alder communities on frost mounds to low lying sedge hollows in ice-wedge polygons. Plot sizes were between 3 × 4 m and 5 × 5 m depending on the community type, and oil was applied at various levels equivalent to 0.25 cm up to 1.50 cm. The second application is equivalent to 1,950 barrels per acre. Evaporation rates were estimated from open containers of oil, and temperature profiles in the peat together with the depth of the active layer were recorded.

Summer evaporation rates of oil were high, up to 15% volume losses being recorded within 16 h of application. Since many of the most phytotoxic components of crude oil are volatile aromatics, one suspects that this reduces the potential harm done by summer spills. On the other hand winter spills may not affect deep roots which are protected by permafrost. All lichens and all mosses except *Polytrichum juniperinum* were killed, and there was no recovery even a full growing season after application. The recovery of the remaining vegetation varied with species and with the level and season of applica-

tion; for example, *Carex* (sedge) species recovered less well following late season applications, whereas *Salix* (willow) species recovered better after autumn spills. In general it was the willows, birches and *Ledum palustre* (Labrador tea) which recovered most effectively from the oil applications. Total plant recovery was occasionally as great as 55% one growing season after the spill, which suggests that recovery of vegetation is best allowed to occur naturally, without further interference.

Temperature measurements showed that areas affected by spills absorbed more energy, so warming superficial layers. Increases in temperature, however, did not occur at great depth within the peat and the depth of the active layer was not affected. Probably much energy was lost in latent heat of evaporation and the drying surface peat would form an insulating cover over the cold, deeper layers.

It seems, then, that the tundra can probably cope with occasional, acute pollution from oil spills. It would be interesting to know whether this ecosystem can also survive low intensity, chronic pollution from the small spills which will inevitably be associated with the exploitation of these fields.

Half life of heavy chain message

from a Correspondent

IN estimating the turnover rate of mammalian messenger RNA there are two main problems—choice of suitable chase conditions and specific isolation of messenger RNA molecules for defined products. The presence of large pools of nucleotide precursors within eukaryotic cells creates obvious problems in a standard pulse-chase type of experiment. Decay of messenger in the presence of a metabolic inhibitor, for example actinomycin, is no longer considered to be satisfactory since the presence of drugs may cause generalised cellular malfunction so that synthesis and processing of RNA within such cells are abnormal.

Cowan and Milstein (*J. molec. Biol.*, 83, 469; 1974) have approached the question with specific reference to the RNA species which codes for the heavy and light chains of an immunoglobulin secreted by a tissue culture-adapted mouse plasmacytoma. The cells were labelled with ³H-uridine continuously during a period of 24 h in an automatic apparatus (see Cowan and Milstein, *Biochem. J.*, 128, 445; 1972), which allows the authors to calculate dilution due to growth, and thus correct the figure for RNA decay during the chase period.

Since no single chase procedure is entirely satisfactory several different conditions were studied. Cells were chased simply by the addition of cold uridine to the medium (the disadvantage of this procedure is continued and significant incorporation of low specific activity uridine during the chase which results in an overestimate of the messenger RNA half life). Alternatively, the cells were removed from the medium containing the isotopic uridine by centrifugation, and then resuspended in fresh medium containing 0, 0.1 or 0.2 M uridine. Exposure of the cells to uridine, however, causes appreciable swelling, and centrifugation is followed by a lag period of 3 h before re-entry into logarithmic growth. The messenger RNA molecules for heavy and light chains were purified by sucrose density gradient centrifugation of the microsomal extract, followed by selection of poly(A)-containing molecules using oligo(T)-cellulose. Purity of the heavy chain messenger was not known, and the light chain mRNA was 30% pure.

When radioactive uridine was removed for the chase period, similar half-lives for both heavy and light chain messenger preparations (12–14 h) were recorded whether cold uridine was present or absent. Thus, although none of these conditions is entirely satisfactory, the fact that the different chase conditions yielded similar results would suggest that the half-life values obtained were meaningful. Not unexpectedly the half-lives were longer (17–18 h) when the chase was done without removal of the radioactive uridine.

All of these values are much longer than those based on immunoglobulin synthesis in myeloma cells grown in the presence of actinomycin, probably indicating the unreliability of the actinomycin chase procedure. As the authors themselves clearly indicate, a crucial point is the purity of the messenger samples. Thus, values in the range 5–20 h cannot be excluded for the light chain message. Until the purity of the heavy chain message preparation is known, it would be premature to accept the half-life given as definitely that of heavy chain message. With increasing refinements in message RNA isolation and characterisation this question should be answered shortly.

Is Viking 1979 a possibility?

from our Cosmology Correspondent

THERE may be enough bits and pieces left over from the planned 1975 Viking missions to Mars to provide a low cost mission in 1979. Less probable, but still being actively considered, is a mission

including a Mars Rover, which could fly in 1979 or 1981 and would again use any spare parts from backups for the 1975 mission.

The 1975 missions will involve two landers and two orbiters, and a third lander is being built as a backup; this lander will, if all goes well, be available for a one-off mission in 1979, when an orbiter could be assembled from spare parts for the Viking 75 orbiters, according to a report in *Aviation Week and Space Technology* (February 11, page 56; 1974). This one-off mission would cost only some \$250 million to \$300 million, including the cost of a new scientific package. If a double mission like that of 1975 were to be attempted, one complete set of orbiter and lander would have to be built from scratch and this would push the total cost up to around \$500 million.

But perhaps the more intriguing possibility is that a Mars Rover vehicle could be added to the mission, at greater cost but without great technical difficulty. The estimated total cost of such a lander is some \$80 million and it is possible that the European Space Research Organisation (ESRO) would participate in this if the project goes ahead. Unfortunately, however, ESRO prefers the 1981 target date and this would involve NASA in much more expense, because of the need to keep Earth-based communications and staff available for another two years.

There is also the problem that NASA would like to do something in 1979 and if the 'spare parts' are used up then in a repeat of the 1975 missions, the cost of a 1981 rover mission would be increased by the need to build the lander and orbiter as well as the rover.

Leaving aside these difficulties for the present, just what would be involved for a lander-orbiter-rover mission in a few years time? The basic rover design suggests a 238-pound vehicle, which would add a total weight of 282 pounds to the Viking lander, including stowage and deployment mechanisms. The Martin Marietta Corporation sees no serious problems in having this vehicle ride piggyback on top of the Viking lander and suggests that this would only involve a slight loss of the lander's other scientific capability. As things now stand, the scientific payload of the rover, including camera, spectrometer, X-ray diffractometer and other experiments, would be 89 pounds.

The total weight of the lander-rover package would be 1,583 pounds; this is 106 pounds more than specifications for the 1975 landers define as safe, but it turns out that the rocket motors of these landers exceed their specification and that the maximum safe weight for the lander-rover could therefore go

up to 1,644 pounds if need be.

As well as making its own investigations over a range of 45 km, such a rover could make itself useful by off loading instrument packages from the lander and carrying them several metres away from the landing point, so that they could be deployed more efficiently.

Since the cost of integrating the rover into the lander system, which could be as much as 25% of the total cost of the rover, would be borne by NASA, it seems that ESRO could make a great contribution to the unmanned exploration of the planets for a cost of about \$60 million. This seems a small price to pay and it is to be hoped that ESRO is indeed taking the project seriously.

Amateur and professional entomology

from a Correspondent

THE entomological *Bulletin of the British Museum (Natural History)* is a handsome production, as befits so old and eminent an institution. Its recent issues, comprising volume 28 parts 6 and 7, and volume 29 parts 1 and 2, as well as supplement 20, provide good samples of the sort of systematic work that goes on within the museum walls. There are two works by members of the museum's staff, two by entomologists from other institutions who have worked on the museum's collections, and one by an amateur lepidopterist to show that an ancient and very English tradition is still upheld. One work provides a list of the numerous type specimens of an important butterfly genus held by the museum, two provide revisions of the species of particular genera, drawing more or less extensively on materials from other museums as well as the British Museum. The two largest parts are concerned with higher level groupings, an aspect of classification which sometimes tends to be neglected in publications of this kind. The two, however, differ profoundly in nature.

C. M. F. von Hayek, of the museum's staff, provides "A Reclassification of the Subfamily Agrypninae (Coleoptera; Elateridae)" (*Bull. Br. Mus. nat. Hist. (Entomology)*, Suppl. 20, 1-309; 1973) and the amateur J. N. Eliot deals with "The Higher Classification of the Lycaenidae (Lepidoptera)" (*ibid.*, 28, 373-508; 1973). Hayek's work is in the best professional tradition; she provides a very full discussion of the complicated synonymy in the group, a clear and workable key to genera and full synonymic catalogues of the species for each genus, with the designation of numerous lectotypes, many of them in foreign museums. She eschews any consideration of larval characters (known for many of

the species she deals with), habits or phylogeny, and is not very generous with illustrations, as compared with the other four authors. Her most questionable classificatory decision is the inclusion in Agrypninae of the genus *Octocryptus*, about which she herself expresses reservations.

Eliot provides no catalogues of species or even of genera, but provides keys down to the level of tribes and sections (= subtribes), and numerous illustrations of male genitalia and wing scales, including six plates of electron micrographs. A large part of his space is taken up with the systematic, adaptive and phylogenetic significances of characters of his butterflies, and he provides an extensive account of their phylogeny, including a dendrogram. The speculative nature of much of this is admitted by Eliot, and it remains for specialists in the group to evaluate his theories. He takes account of larval and pupal characters, geographical distribution and palaeogeography, but pays little attention to food plants (Ehrlich and Raven's celebrated *Butterflies and Plants: a Study in Co-evolution* is not even cited in his bibliography). Eliot's paper is designed to evoke thought and curiosity about his lycaenid butterflies, a result which Hayek is not likely to achieve for the Agrypninae, for all the usefulness her work will have for those concerned with the identification and arrangement of museum collections of the group. On the other hand, one feels that Eliot's work might gain from some injection of Hayek's formal procedural rigour, as she would certainly gain from his amateur breadth of outlook and intellectual curiosity.

New IR telescope

Buried in NASA's budget for 1975 is a little-noticed item which holds particular interest for astronomers. NASA is planning to build a large infrared telescope on Mauna Kea in Hawaii, some 14,000 feet above sea level. With a 3-m dish, it will be the largest infrared telescope in the world.

The chief reason for building the telescope—and the reason it is in NASA's budget rather than in that of the National Science Foundation—is to use it to get information on Jupiter, Saturn and their satellites in time for the 1977 Mariner Jupiter-Saturn flyby missions. Temperature profiles of the moons of those planets will be helpful in targeting the spacecraft to take a look at the most interesting features. Ultimately, however, the instrument, which will cost about \$6 million, will be used for general infrared studies of both galactic and extragalactic objects.

Infectious C-type virus isolated from a baboon placenta

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A C-type virus ("M7") has been isolated from a specimen of baboon placenta cocultivated with various mammalian host cell lines. Nucleic acid hybridisation with a ³H-DNA transcript of the viral genome demonstrates that this virus is genetically different from previously studied mammalian C-type viruses, and suggests that M7 is an endogenous baboon virus.

C-TYPE RNA-containing viruses have been isolated from tissues and cell lines of various mammalian species¹, including mice²⁻⁴, rats⁵, hamsters⁶, guinea pigs⁷, domestic cats⁸, pigs^{1,9}, woolly monkeys¹⁰ and gibbons¹¹. Because of the known aetiological role of these viruses in the generation of vertebrate neoplasms¹², there have been extensive attempts to isolate infectious C-type viruses from other primates, including man. Recent electron microscopic evidence indicates that C-type viruses are present in the placentas of baboons¹³ and rhesus monkeys¹⁴, and, less frequently, human trophoblastic tissue¹⁵. We describe here the isolation and *in vitro* propagation of a typical C-type virus from a normal baboon placenta. Nucleic acid studies suggest an endogenous baboon origin for this virus isolate and demonstrate that it is distinct from known described mammalian C-type viruses, including two of presumed primate origin.

First trimester placental tissue was obtained by Caesarian section from an 11-yr-old multiparous baboon (*Papio cynocephalus*) from the colony maintained at the Southwest Foundation for Research and Education, San Antonio, Texas. This animal was imported to the United States from Africa in 1968 and had not been used previously for medical research. A portion of the placenta was removed for viro-

logical studies and the remainder was processed for nucleic acid extraction. Small pieces of the placenta were cocultivated directly with the indicator cell lines described in Table 1. In addition, a 5 g sample was homogenised in a Teflon pestle tissue grinder, suspended in 15 ml of cell culture medium, and centrifuged at 1,000g for 20 min. The supernatant was filtered through a 0.45 µm Millipore filter, and used to infect various mammalian cell lines, some of which are highly permissive for endogenous mouse and cat C-type viruses^{16,17}. The cultures were transferred every 2 weeks, and, in most cases, by the third transfer there was no evidence of baboon placental cells remaining in the cocultivated cultures.

Cultures were tested for C-type virus production by assaying for viral reverse transcriptase in the tissue culture media¹ (Table 1). After 5 weeks of cocultivation, C-type viral replication was first detected in FCf2Th, a foetal canine cell line. By 13 weeks, viral replication could also be detected in the rhesus monkey cell line, DBS-FRHL-1, in the bat lung cell line, Tb1Lu, and in the human rhabdomyosarcoma line, A204. Viral replication was also detected after 13 weeks in the cultures of A204, DBS-FRHL-1 and Tb1Lu which received filtered extracts of the baboon tissue (Table 1). The four other cell lines remained virus-negative during the 3-month test period.

The polymerase-positive foetal canine cell culture, FCf2Th, was examined by electron microscopy. As Fig. 1 shows, typical C-type viral particles were present in the extracellular space and in intracytoplasmic vacuoles, and were seen budding from the cell membranes. Designated M7, this virus rapidly generated productive infections of previously virus-free cultures of A204, DBS-FRHL-1, Tb1Lu, the mink cell line Mv1Lu, the dog cell line MDCK and the equine cell line E. Derm. No viral replication was detectable in rat NRK cells or in a foetal cat strain, FFc60WF,

TABLE 1 Growth of Baboon Placental Virus on Various Mammalian Cell Lines

Cell line (or strain)	Origin	Supernatant reverse transcriptase assay (c.p.m. × 10 ⁻³ , ³ H-TMP incorporated)					Days after infection with filtered extract	
		15	23	36	60	90	60	90
A204	Human rhabdomyosarcoma ²¹	0.9	3.5	10.0	20.7	749.8	764.3	810.0
A1215	Gibbon connective tissue	0.4	0.7	1.4	NT	1.8	1.1	NT
DBS-FRHL-1	Foetal rhesus lung ²²	2.6	3.2	1.7	33.8	1025.6	4.3	1080.2
FCf2Th	Foetal canine thymus (NBRL)	1.1	1.0	209.6	227.7	NT	NT	NT
SIRC	Rabbit cornea (ATCC)	0.6	0.6	2.7	5.6	2.9	3.9	5.3
Tb1Lu	Bat lung (ATCC)	0.8	0.6	1.5	4.5	762.6	4.0	337.0
FFc60WF	Foetal cat (NBRL)	1.6	1.5	1.5	3.3	2.3	NT	NT
Bu(IMR-31)	Buffalo lung (ATCC)	1.0	2.1	2.1	2.4	1.5	1.1	2.5

Cell culture medium was assayed at various times after cocultivation or infection for reverse transcriptase activity as previously described¹. Results are expressed as c.p.m. of ³H-TMP incorporated into poly(dT) product during a 60 min incubation at 37°C (40,000 c.p.m. minute is equivalent to 1 pmol of ³H-TMP). ATCC, American Type Culture Collection, Rockville, Maryland; NBRL, Naval Biomedical Research Laboratory, Oakland, California.

NT, not tested.

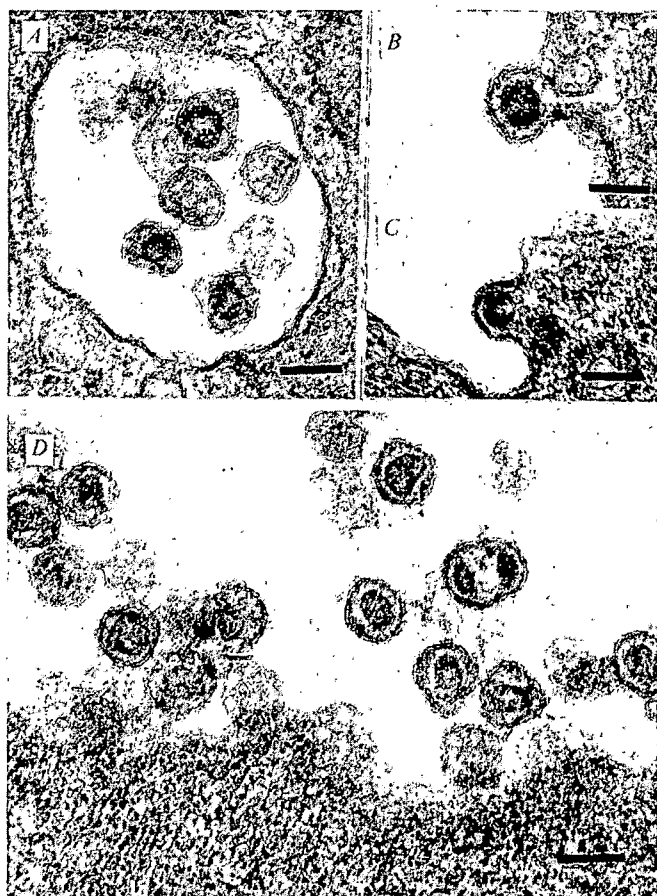


Fig. 1 Electron micrographs of C-type virions released by a foetal canine thymus cell line (FCf2Th) infected with an extract from a baboon placenta. Cell monolayers were fixed with buffered 2% glutaraldehyde and then scraped off with a rubber policeman. Cells were then sedimented and fixed in 1% buffered osmium tetroxide. After overnight incubation in cold 0.5% uranyl acetate, the cells were dehydrated and embedded in Epon. Thin sections were doubly stained in uranyl acetate and lead citrate and examined with a Siemens Elmiskop 1A electron microscope. A, intracellular particles. B and C, particles budding from the foetal canine cells. D, extracellular C-type particles. The scale represents 100 nm.

that is permissive for both feline leukaemia virus and endogenous feline C-type viruses (RD114/CCC group)¹⁶.

M7 virus was grown in FCf2Th cells, concentrated by continuous flow centrifugation, and further purified by isopycnic banding on sucrose gradients. The peak of reverse transcriptase activity occurs at a density of 1.16 g cm⁻³, characteristic of C-type viruses. Banded virus was disrupted with detergent and chromatographed on a Sephadex G-100 column calibrated with several protein markers. The reverse transcriptase activity eluted at a position corresponding to a molecular weight of 70,000, similar to that reported for other mammalian C-type viral polymerases¹⁸. Enzymatic activity assayed with a poly(A) template and oligo (dT)₁₂₋₁₈ primer showed the divalent cation preference for Mn²⁺ over Mg²⁺, characteristic of mammalian C-type viruses¹⁹.

The relatedness of the M7 virus to other C-type viruses was determined by comparing nucleic acid homology using DNA-RNA hybridisation. A single-stranded ³H-DNA copy of M7 viral RNA prepared using endogenous RNA-dependent DNA polymerase in the presence of actinomycin D²⁰ was annealed to viral RNAs or to cytoplasmic RNA extracted from cell lines that release C-type virus. Table 2 shows representative data obtained after digestion of the RNA-DNA hybrids with the single-strand specific nuclease,

S₁²⁰⁻²². The data have been normalised with respect to the final percentage saturation hybridisation values obtained with M7 viral RNA; these values ranged from 50-60%. Interpretation of RNA-DNA hybridisation using ³H-DNA prepared from an endogenous reverse transcriptase reaction is limited since the probe need not represent equally the entire viral RNA²³. Thus, homologies between infrequently transcribed portions of the M7 genome and other viral genomes may not be detected. Nevertheless, the data show that viral RNA extracted from three murine viruses, a rat virus, a feline virus, a Chinese hamster virus, a pig virus, and two presumed primate viruses (woolly monkey and gibbon) have less than 2.0% homology to the ³H-DNA probe prepared from M7. The probe hybridised to a small extent (10%) with an endogenous feline virus, RD114; the significance of this relationship is not yet clear. These data

TABLE 2 Nucleic Acid Homology of the M7 ³H-DNA Product to other C-type Viral RNAs

C-type virus	Origin	Percent M7 ³ H-DNA hybridised
M7	Baboon placenta	100.0
Gibbon	Gibbon lymphosarcoma	1.5
Woolly	Woolly monkey sarcoma	2.0
Feline	Cat leukaemia (Rickard)	1.8
	Human rhabdomyosarcoma cell line passaged through kittens (RD114)	10.0
Murine	Mouse leukaemia (Rauscher)	0.0
	Transformed mouse cell line (S16CL2)	0.5
	Human rhabdomyosarcoma cell line passaged through NIH Swiss mice (AT-124)	0.0
Chinese hamster	Hamster peritoneal cell line (B14-I50)	1.4
Rat	Rat thymus cell line (RT21c)	2.0
Porcine	Pig kidney cell line (PK-15)	0.0

Approximately 2000 c.p.m. (0.07 pmol of ³H-TMP incorporated into DNA) of a single-stranded M7 ³H-DNA product was hybridised to either viral or cytoplasmic RNA²⁰. The data shown represent the averages of final saturating percentage values obtained after adding increasing amounts of viral RNA (up to 20 µg) or cytoplasmic RNA (up to 2 mg) extracted from a virus-producing culture. The percentage hybridisation values have been normalized with respect to the final percentage hybridisation obtained with the homologous M7/FCf2Th RNA (actual values ranged from 50-60%). Hybridisation was carried out for 72 h at 41°C in the presence of 38% formamide as previously described²⁰. The formation of ³H-DNA-RNA hybrids was detected with S₁ nuclease²⁰⁻²². RNA extractions were performed as described previously^{20,23}. S16CL2, PK-15, B14-I50 and RT21c are endogenous viruses spontaneously released from their respective mouse, pig, Chinese hamster and rat cell lines. The Rickard strain of feline leukaemia virus was grown in a cat thymus cell line (F422), Rauscher murine leukaemia virus in the murine line JLSV-9, RD114 and AT-124 in a human rhabdomyosarcoma cell line (RD), gibbon and woolly monkey leukaemia viruses in the human rhabdomyosarcoma cell line (A204), and M7 in the foetal canine thymus cell line, FCf2Th. These viruses and their host cell lines have been described previously²⁴. Viral 70S RNA was extracted²⁵ from gibbon, woolly monkey, feline and murine viruses. The source of rat, porcine, Chinese hamster and M7 viral RNA was cytoplasmic RNA extracted from cultures producing these viruses. Both sources of RNA have been shown to yield similar data²³.

are also obtained when a reverse transcript prepared from each of the viruses listed in Table 2 is annealed to M7 viral RNA. Immunological studies of M7 viral reverse transcriptase and group-specific protein also demonstrate that the virus can be distinguished from known C-type virus isolates. These results argue against the possibility that the M7 isolate resulted from inadvertent infection of the animals, specimens or cell cultures by known laboratory C-type viruses.

To ascertain whether M7 could be an endogenous baboon virus, a single-stranded ³H-DNA copy of M7 RNA was

annealed to DNA extracted from a normal baboon liver. As Table 3 shows, the M7 probe hybridised fully to the DNA extracted from baboon liver and from the placenta from which the virus was isolated. Thus, sequences in the DNA of the liver and placenta from two baboons are completely homologous to those found in the M7 virus that was grown in dog cells. Tissues from four other baboons also contain DNA homologous to the M7 viral DNA probe (unpublished experiments). In contrast, DNA extracted from dog and pig liver, from a murine cell culture producing mouse C-type virus (S2CL3), and from the uninfected foetal canine cell line FCf2Th hybridised to less than 6% of the probe. There is a small amount of M7-related information in feline liver DNA (15%); the nature of this feline-baboon viral homology is examined in a separate report.

Cytoplasmic RNA extracted from the normal baboon liver and from the baboon placenta from which M7 was isolated was tested for its ability to anneal to the M7 ³H-DNA probe. Table 4 shows that whereas M7-specific viral RNA is readily detectable in baboon placenta, the addition of up to 0.9 mg of RNA from the baboon liver resulted in less than 2.6% hybridisation to the M7 probe. Thus, M7 viral information, though present in the cellular DNA, is not extensively transcribed in normal baboon liver tissue.

C-type viruses have been observed by electron microscopy in baboon placental tissue, and the hybridisation data demonstrate that RNA homologous to the M7 virus genome is being produced. In contrast, viruses have not been seen in adult baboon liver, and M7 viral RNA is not detectable by nucleic acid hybridisation. Thus, while viral genetic information is present in the DNA of both a virus-producing tissue (the placenta) and an apparently virus-free tissue (the liver) only the placental tissue is 'switched on' for virus production. The physiological basis for control of C-type virus expression and the mechanisms that allow virus activation in placental tissue remain to be elucidated.

These findings suggest that M7 is an endogenous C-type virus of the baboon. Similar nucleic acid hybridisation experiments have previously shown that chickens²⁴⁻²⁶, cats²⁷⁻²⁹ and pigs³⁰ also have vertically transmitted C-type viral genomes. Since ³H-DNA probes prepared from the woolly monkey virus and the gibbon ape virus show little or no homology to the DNA extracted from normal woolly or gibbon tissues (Scolnick *et al.*, manuscript submitted; Ben-

TABLE 3 Hybridisation of M7 ³H-DNA to DNA extracted from Tissues of Various Species

Species	Tissue	Percentage M7 ³ H-DNA hybridised
Baboon	Placenta	100.0
	Liver	115.0
Canine	Liver	5.0
	Cell culture (FCf2Th)	6.0
	Cell culture (FCf2Th)	100.0
	(M7 infected)	
Feline	Liver	15.0
Porcine	Liver	1.0
Murine	Cell culture (S2CL3)	2.0

The data show normalised percentage hybridisation values to the M7 ³H-DNA probe obtained after annealing to cell DNA to a *Cot* value of 8×10^3 (corrected for salt concentration to 0.12 M phosphate as described by Britten and Smith³⁴). DNA-DNA hybridisations were performed as previously described³⁰; the hybrids were detected with S₁ nuclease. DNA extractions were performed as described previously³⁰. The ratio of cell DNA (300 µg) to ³H-M7 DNA (0.1 ng) was 3×10^6 .

veniste *et al.*, manuscript submitted), M7 seems to be the first example of a vertically transmitted C-type virus of primates.

Our experiments demonstrate the validity of a general strategy for *in vitro* isolation of C-type viruses involving

cocultivation of suspected virus-producing tissues with cells from various species to maximise the probability of finding a permissive host. The same general approach may be applicable in attempts to isolate infectious C-type viruses from primates other than the baboon and from tissues other than the placenta.

TABLE 4 Expression of M7 Virus-specific RNA in Baboon Liver and Placental Tissue

Tissue	µg RNA added	Percentage M7 ³ H-DNA hybridised
Placenta	94	12.0
	282	22.5
	940	40.5
Liver	90	1.8
	450	2.6
	900	2.4

Approximately 1,600 c.p.m. of a single-stranded M7 ³H-DNA probe was hybridised to increasing concentrations of cytoplasmic RNA extracted from the baboon placenta from which M7 was isolated and from a normal liver of another baboon. The percentage hybridisation values have been normalised with respect to the final percentage hybridisation obtained with RNA extracted from the M7-infected foetal canine cell culture, FCf2Th; this value ranged from 50-60%. See legend to Table 2.

Dr A. Hellman helped in these studies; D. Stuart and M. Sturm prepared the electron micrographs. We thank R. Heinemann, C. Meade, C. Meyer and G. L. Wilson for help with the experiments. This work was supported in part by contracts from the National Institutes of Health Special Virus Cancer Program.

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Three-dimensional structure of yeast phenylalanine transfer RNA at 3.0 Å resolution

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At 3 Å resolution, the electron density map of crystalline tRNA shows the polynucleotide chain as an alternating series of ribose and phosphate peaks. Bases are seen, especially in the double helical stem regions. A complete three-dimensional model of the L-shaped molecule has been built.

TRANSFER RNA is the molecule on which the polypeptide chain is assembled when tRNA interacts with mRNA. There is considerable interest in the three-dimensional structure of this molecule as a first step in understanding the detailed mechanism of protein synthesis. For several years we have been analysing crystals of yeast phenylalanine tRNA. The molecule crystallises in an orthorhombic unit cell ($P2_12_1$, $a = 33\text{Å}$, $b = 56\text{Å}$, $c = 161\text{Å}$) and these crystals produce high resolution X-ray diffraction patterns¹. We obtained heavy atom derivatives which made it possible to calculate an electron density map initially at a resolution of 5.5 Å² and at 4.0 Å resolution³. From the latter study it was possible to discern the overall shape of the molecule and show that the molecule was L-shaped with one arm of the L containing the CCA and TΨC stems while the other arm contained the dihydrouracil (hU) and anticodon stems which are components of the familiar cloverleaf arrangement of the polynucleotide sequence of yeast phenylalanine tRNA⁴ (Fig. 1). At 4 Å resolution the ribose-phosphate groups could be seen as individual peaks and the tracing of the chain was carried out largely by following adjacent peaks in the electron density map. Now we report that at 3 Å resolution the phosphate groups are typically seen as individual peaks separated from the ribose peaks. In addition, many of the purine and pyrimidine bases can be seen, especially in those regions in which base pairs are formed. The tracing of the polynucleotide chain seen at 3 Å resolution is virtually the same as that seen at 4 Å resolution, but many more details begin to emerge.

Structure and electron density

The experimental details for crystal growing and heavy atom derivative formation as well as data collection have been described previously^{1,2}. We have explored more fully

the conditions under which heavy atom derivatives can be formed and have developed several more heavy atoms sites, whose positions in the unit cell are listed in Table 1. The most important of these are the samarium and osmium derivatives. There are four samarium sites, two of which have major occupancies and two of which are less fully occupied. The osmium derivatives have in addition to the major site described previously², two sites with lesser occupancies. A double derivative has been obtained by soaking crystals initially with potassium osmate and then with samarium acetate. In addition, we have platinum de-

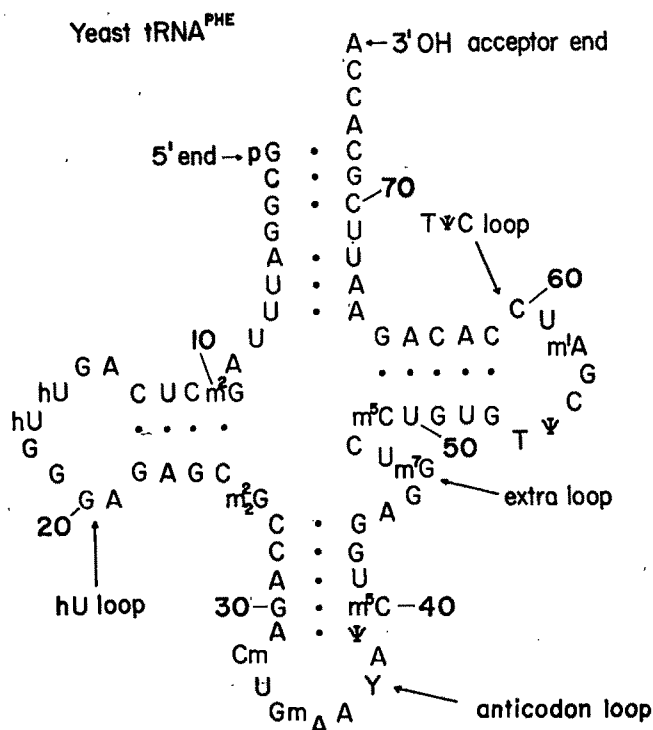


Fig. 1 The nucleotide sequence of yeast phenylalanine tRNA in the conventional cloverleaf diagram⁴. The nucleotides are numbered starting from the 5' end.

derivatives containing four sites and praseodymium derivatives with the same four sites as those found in the samarium derivatives.

The 3 Å sphere contains 6,807 unique reflections for this unit cell and of these, 4,902 were used in calculating the three-dimensional electron density map. Bijvoet pair data was collected for the derivatives. The overall figure of merit is 0.66 for the data used and the various residuals for each of the different derivatives are listed in Table 2. It can be seen that the most useful data was that produced by the samarium and osmium-samarium derivatives. The platinum data had only limited value and the osmium data was useful likewise only at low resolutions. A full crystallographic report will be published elsewhere.

Three-dimensional electron density maps were plotted at intervals of approximately 0.8 Å along the *a*, *b* and *c* axes. The map sections perpendicular to the *b* axis were used in assembling molecular models because of the more ready visualisation of the molecule. The electron density contours were drawn on glass or plastic sheets at a scale of 2 cm Å⁻¹ which were then mounted in a Richard's box⁵.

TABLE 1 Heavy atom parameters

	X	Y	Z	Occupancy*
Sm (3.0Å)	0.555	0.169	0.275	M
	0.279	0.373	0.232	M
	0.849	0.105	0.215	I
	0.188	0.175	0.157	W
Os-Sm (3.0Å)	0.558	0.167	0.275	I
	0.282	0.372	0.232	M
	0.867	0.106	0.214	I
	0.198	0.179	0.160	W
Os (4.0Å)	0.035	0.196	0.065	I
	0.029	0.193	0.066	M
	0.006	0.274	0.231	I
	0.073	0.234	0.051	I
Pt (4.0Å)	0.040	0.189	0.061	I
	0.198	0.148	0.036	I
	0.105	0.224	0.324	I
	0.244	0.417	0.114	I
Pr (5.0-4.0Å)	0.571	0.168	0.280	I
	0.277	0.376	0.233	I
	0.829	0.101	0.215	I
	0.187	0.172	0.158	I

* Occupancy: M, major site; I, intermediate site, W, weak site.

Kendrew wire models were used to build the molecule. The electron density map showed the gross outlines which were described previously in the 4 Å map. In addition, the 3 Å map revealed much more detail than was available at 4 Å. Substantial regions of the map are occupied primarily by solvent and there is very little accumulation of electron density in these areas. A channel of approximately 36 × 40 Å runs parallel to the *a* axis throughout the crystal and it permits the cell shrinking phenomena described earlier⁶. The double helical stems which were recognisable at 4 Å resolution now become clearly resolved; ribose and phosphate groups can be readily discerned from each other over most of the stem regions and the characteristic zigzag array of these two groups provide the continuity needed for tracing the backbone of the molecular chain. Figure 2 shows a photograph of a Kendrew wire molecular model built to fit the electron density in the map. In this view the anticodon is in the upper left and the CCA stem is in the upper right with the TΨC and hU loops forming the corner at the bottom. The L-shaped form of the molecule is readily apparent. The entire asymmetric unit is shown in this view with the exception of three terminal nucleotides CCA at the 3'OH end. The CCA end of one molecule extends into the neighbouring unit cell where it rests close to the anticodon stem of an adjacent molecule. It was not included in Fig. 2 to increase the clarity of the photograph. The distance

between the 3' hydroxyl group at one end of the L-shaped molecule and the anticodon bases at the other end of the L is 77 Å.

TABLE 2 Refinement statistics

	Sm	Os-Sm	Os	Pt	Pr
N	4843	3309	2773	1943	556
Resolution(Å)	3.0	3.0	4.0	4.0	5.0-4.0
R_K	0.094	0.110	0.194	0.310	0.087
R_M	0.63	0.73	1.18	2.52	0.74
R_W	0.49	0.68	1.53	7.76	0.72

N, number of reflections; the residual values R_K , R_M and R_W are defined in ref. 2.

Chain tracing

In attempting to trace the chain from the electron density map, several criteria and constraints were used. The first constraint is the fact that the polynucleotide backbone can be extended to a maximum repeat distance of approximately 7.5 Å or a minimum distance of approximately 4.5 Å. Second, the chain needs connectivity, that is, it should be traceable over its entire length without any gaps in the electron density map. Third, there must be a relationship between the total electron density in the map and the length of polynucleotide chain which is used to interpret the map. All or most of the high density regions in the map should be accounted for. Fourth, the polarity of the polynucleotide chain can be determined in double helical regions. This is due to the fact that these regions show both major and minor grooves in the electron density map. In interpreting these maps, we used conformational data from the fibre diffraction analysis of double helical RNA⁷ as well as from the recent single crystal analyses of fragments of RNA double helix^{8,9}. These studies provided models for interpreting the right-handed double helical stem segments and they unambiguously fix the direction of the polynucleotide chain.

In regions of the map which do not contain double helical segments, however, the connectivity and polarity of the chain is more difficult to determine. In these regions we try to recognise the base peaks and utilize conformational information obtained from the single crystal structure analysis of oligonucleotides⁸⁻¹¹. In addition, we have used the secondary structure implicit in the cloverleaf formation shown in Fig. 1

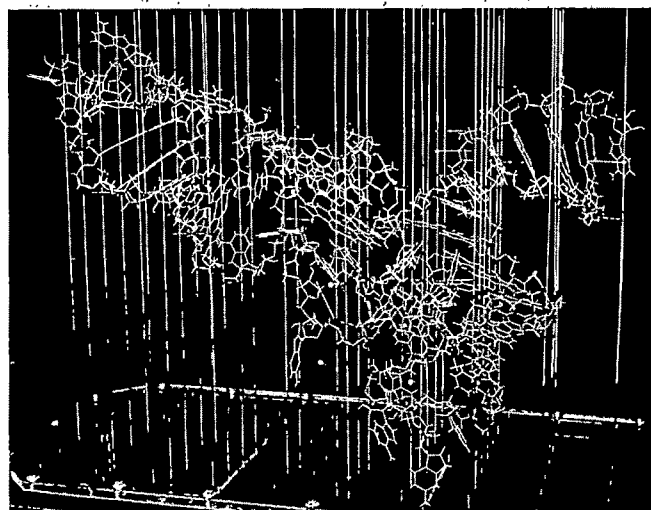


Fig. 2 A photograph of a Kendrew wire model of yeast phenylalanine tRNA based on the 3 Å resolution electron density map. The model is built at a scale of 2 cm Å⁻¹. The CCA stem is at the upper right. The TΨC and hU loops are at the bottom and the anticodon loop is at the upper left. The L shape of the molecule is evident.

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as an adjunct for the chain tracing. Although the map contains double helical stem regions in which the chain tracing is unambiguous, there are areas in which the chain tracing is less clear. In particular, the TVC and hU loop areas are close together near the corner of the L and both chains follow a fairly irregular course. Furthermore, there are additional peaks which we interpret as tightly bound cations. This makes the chain tracing in these areas much less certain than in the double helical stem regions.

As mentioned above the three nucleotides CCA at the 3'OH end of the molecule extend out from the CCA stem and are adjacent to the anticodon stem of a neighbouring molecule. The electron density peaks are slightly lower in the CCA region of the molecule than elsewhere, suggesting somewhat greater disorder in that part of the molecule. The 3' terminal ribose peak is found 3 Å from the major osmium site, in agreement with our suggestion that this site may be due to the formation of an osmium-bipyridine complex with the two *cis* hydroxyl groups of the 3' ribose³. But as we have found two additional osmium sites, it is clear that this is not the sole mechanism by which osmium can complex to RNA molecules. In this regard it is likely that the osmium derivative reported by Schevitz *et al.*¹² represents an alternative mode of complexing.

The three-dimensional Kendrew model was built first by assembling the double helical segments while paying attention to the geometry of these segments as discussed above. A very long double helical segment containing approximately one turn of the helix is seen at one end of the molecule (right side of Fig. 2), the 3' end of which is attached to the isolated segment of four nucleotides which we interpret as the ACCA end. Once the double helical stem segments were built, the identification of loops was guided by the cloverleaf diagram. In general, we are reasonably confident about the assignment of residues in the double helical stem segments. We are not as confident of the loop areas, however, since there are often ambiguities regarding continuity where there are many close contacts.

Figures 3, 4 and 5 show three sections of the electron density map perpendicular to the *a*, *b* and *c* axes. These maps are presented to illustrate the kind of resolution seen in the electron density map, and at the same time the superimposed heavy black lines illustrate our interpretation of those regions of the map. Figure 3 shows a slice, perpendicular to the *c* axis, which passes through one strand of the anticodon stem. It can be seen that there is a reasonable resolution of ribose and phosphate peaks and two of the base pairs in the stem region lie almost in the plane of this slice. Although the base pair can be seen in the map, it is clear that we cannot differentiate one base from another at this resolution. There are sections of electron density in the upper right and lower right of Fig. 3 which arise from neighbouring molecules.

A section perpendicular to the *b* axis is shown in Fig. 4. The large empty area to the left is the region filled largely by solvent and it is this region which disappears when the crystals are transformed into a smaller unit cell by shrinkage⁶. This section contains an elongated length of polynucleotide chain including part of the hU stem region. The zigzag nature of the ribose phosphate chain can be seen fairly clearly. The vertical *a* direction contains one and a half unit cells because the molecule itself spans more than one unit cell in the *a* direction. All the labelled residues in Fig. 4 are in one molecule. The unlabelled parts are related to the labelled parts by symmetry, either a translation in the vertical *a* direction or a two-fold rotation as indicated by the axes shown in the figure. The peak labelled M is interpreted as a metal ion which is found in the position where two molecules are in close contact.

Figure 5 shows a section perpendicular to the *a* axis. The

arc of electron density seen at the left of the figure is a portion of the TVC stem which lies in the *bc* plane. The section at the far right cuts through the anticodon part of the molecule one unit cell below whereas the portion in the middle which contains very little electron density lies between two molecules.

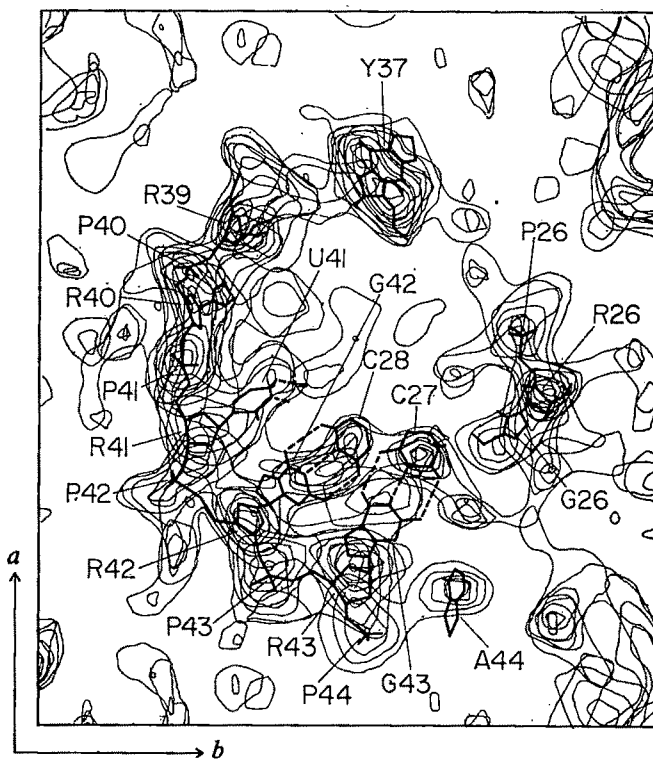


Fig. 3 A composite of electron density map sections cut perpendicular to the *c* axis in the interval $z = 0.110-0.120$. The vertical distance is slightly greater than an *a* axis repeat of 33 Å. The horizontal axis is close to one half of the 56 Å *b* axis. The heavy lines represent portions of the molecule, and a part of the anticodon stem is seen. The electron density at the upper right and lower right arise from adjacent molecules in the next unit cell. P, phosphate group; R, ribose.

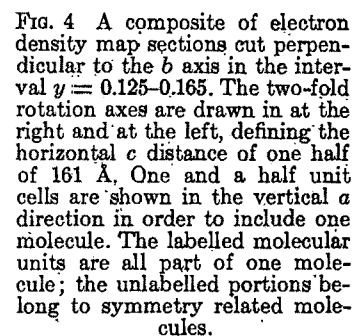
As is obvious from Figs 3, 4 and 5 most of the peaks of electron density are accounted for by structural features of the molecule. Some peaks, however, are not accounted for by the polynucleotide backbone. Many of these are believed to be ions, especially since a number of them are found within 4 Å of the negatively charged phosphate groups. In addition to the magnesium ions, the solvent also has the arsenic-containing cacodylate ions of the buffer as well as the polycationic spermine. Some additional experiments are necessary in order to ascertain the nature of these additional peaks in the electron density map.

Molecular structure

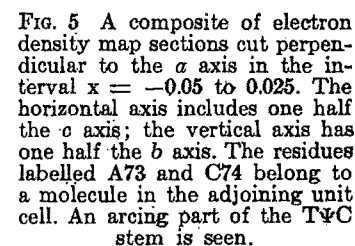
There are a few important features of the connections between the stem areas that should be described. In going from the CCA stem to the hU stem there are two nucleotides, 8 and 9, where the chain makes an abrupt change in direction. The uracil in position 8 was found to be partially stacking over the cytosine of nucleotide 13; thus the photodimerisation reported by Yaniv *et al.*¹³ can easily be explained by this juxtaposition. We have made a reasonable tracing of the hU loop in which the guanine of nucleotide 15 forms a hydrogen bonded pair with the cytosine of nucleotide 48. This base pair has been described as a possible feature of the secondary structure of tRNA¹⁴. However, since this occurs in a loop region, we must still regard this as somewhat tentative.

The electron density in the CCA stem shows only a slight irregularity in the region of the guanine-uracil base pair; thus we can say that these bases are not folded out of the stem, but rather are found stacked within the double helical portion of the molecule. Further work, however, is needed to show whether or not these bases are hydrogen bonded in the stem.

The diffraction pattern of this orthorhombic unit cell extends out to a resolution of 2.3\AA , thus almost twice as



much diffraction data can be collected by extending the study from 3Å to 2.3Å. We are continuing the analysis in this direction and are also continuing the search for additional heavy atom derivatives which will help to define further the phases used in calculating the three-dimensional electron density map. At present we are confident of the overall form of the molecule and can define in reasonable detail the



double helical stem regions. The loop areas, however, remain somewhat uncertain and although we can make quite reasonable chain tracings we cannot be certain that these chain tracings are unique. This is of particular importance in that we would like to define completely the conformation of the hU loop as well as the detailed and interesting interaction between the T Ψ C and hU loop areas in the corner of the molecule. We hope that these will emerge in our continuing study.

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Morphine-like drugs inhibit the stimulation by E prostaglandins of cyclic AMP formation by rat brain homogenate

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In homogenate of rat brain, morphine, at concentrations obtainable in vivo, inhibited the stimulation by prostaglandin E₁ or E₂ of cyclic AMP formation, without inhibiting the basal production of cyclic AMP. Heroin was more and methadone less active than morphine, whereas dextrorphan was inactive and naloxone antagonised the effect of morphine. This inhibition may represent a mechanism whereby morphine-like drugs exert their analgesic or other effects.

EXPERIMENTAL administration of E prostaglandins elicits the main pharmacological effects: pain or hyperalgesia^{1,2,3}, diarrhoea^{4,5} and cough^{6,7}, that opiates are clinically used to lessen⁸. This antithesis extends to other effects of E prostaglandins and opiates⁹⁻¹³. Reports have also appeared of antagonism between morphine and E prostaglandins in some isolated intestinal preparations¹⁴⁻¹⁷ and in the release of adrenocorticotrophic hormone^{18,19}. Such considerations led us to examine whether morphine might inhibit some biochemical process of E prostaglandin (PGE) production or action at appropriate concentrations *in vitro*. The finding of Vane²⁰ that morphine does not inhibit biosynthesis of PGE₂ was confirmed, using rat brain homogenate with glutathione and hydroquinone as cofactors (S. A. Saeed, P. J. Gardiner and H. O. J. C., unpublished). Rat brain homogenate, however, converts adenosine triphosphate (ATP) to cyclic 3',5'-adenosine monophosphate (cyclic AMP) through the action

of adenylyl cyclase. We report that PGE₁ or PGE₂, but not PGF_{2 α} , stimulated the formation of cyclic AMP from ATP in rat brain homogenate and that morphine-like drugs potentially inhibited this stimulation without inhibiting the basal production of cyclic AMP in the absence of E prostaglandin.

Measurement of cyclic AMP formation

In preparing brain homogenate, Tris-HCl buffer (50 mM, pH 7.4), containing 250 mM sucrose, 1 mM MgCl₂, 25 mM KCl, 25 mM NaCl, 0.1 mM EDTA and 1.3 mM 2-mercaptoethanol, was used. The buffer was chilled to 0-2° C and operations were done whenever possible at this temperature. Rats (90-120 g) were decapitated, the whole brain was quickly removed, washed in buffer and transferred to fresh buffer. The brain was chopped and then gently homogenised in ten volumes of buffer in a Jencon glass homogeniser.

The formation of radioactive cyclic AMP from its labelled precursor, ³H-ATP, was measured at a total concentration of ATP in the reaction mixture of 1.16 μ M. An excess of non-radioactive cyclic AMP was included in the reaction mixture to protect the newly formed radioactive cyclic AMP from degradation by phosphodiesterase²¹. Brain homogenate (0.05 ml) was incubated with 0.75 ml of a mixture containing Tris-HCl (pH 7.4, 50 mM); MgCl₂, 2 mM; KCl, 10 mM; NaCl, 10 mM; bovine serum albumin, 0.015%; cyclic AMP, 3 mM; ³H-ATP (21 Ci mmol⁻¹), 2.5 μ Ci;

unlabelled ATP, 1 μ M; with or without E prostaglandin (0.25–50 μ g per tube) and drug at concentrations stated in the text. The drugs used were (–)morphine sulphate, (–)heroin hydrochloride, (\pm)methadone hydrochloride, dextrorphan tartrate and (–)naloxone hydrochloride, the concentrations of drug being expressed as active base. Incubations were carried out in duplicate at 30° C for 20 min in a Gallenkamp shaking incubator and were terminated by immersion in boiling water for 3 min after the addition of 2 mM cyclic AMP and 2 mM ATP. After freezing and thawing, the tubes were centrifuged for 15 min at 2,500g. Reaction blanks consisted of incubation tubes containing previously boiled supernatant fractions.

The labelled cyclic AMP formed was isolated by chromatography on Dowex 50W-X4 columns followed by a double Ba(OH)₂-ZnSO₄ precipitation²², care being taken that the pH of the final supernatants did not exceed 8 (ref. 23). The radioactivity contained in 1 ml aliquot of the final supernatants was measured in a Beckman LS-150 liquid scintillation spectrometer.

Blockade of PGE stimulation

In five experiments, addition of PGE₁ (50 or 25 μ g per 0.8 ml tube of incubate) increased cyclic AMP formation by 1.4–2.7 times the basal production in the control tubes (without added prostaglandin). In one experiment, PGE₁ was added to incubation tubes in amounts that were reduced tenfold. The stimulation by PGE₁ of cyclic AMP formation over that in control tubes was: 25 μ g PGE₁, 2.7 \times ; 2.5 μ g PGE₁, 1.2 \times ; 0.25 μ g PGE₁, 0.92 \times . In both of two experiments, PGE₁ was about equal to PGE₂ in stimulant activity. In one experiment in which PGE₂ was tested at 25 μ g per tube, this prostaglandin was inactive.

In five of five experiments, morphine inhibited the stimulation by PGE₁ of cyclic AMP formation by rat brain homogenate, without inhibiting the basal production of cyclic AMP (Fig. 1). The potency of morphine was high and dose-related. For example, in the experiment of Fig. 1, the 50% inhibitory concentration (IC₅₀) was 1.46 μ g ml⁻¹ (95% fiducial limits, 1.20–1.82) and the slope of the dose/response line based on percentage reduction of responses to PGE₁ per log₁₀ concentration of morphine was 61.90 with s. e. \pm 2.8. This slope was highly significant ($P < 0.001$). In another experiment, in which PGE₂ was used instead of PGE₁, morphine showed activity comparable with that against PGE₁ (IC₅₀, 2.03 μ g ml⁻¹).

When the amount of PGE₁ used to stimulate cyclic AMP formation was reduced ten-fold, from 25 or 50 to 2.5 or 5 μ g per tube, the effectiveness of morphine was raised (Fig. 2). In this figure, based on two experiments, the mean slope of the line relating the inhibitory effect of a given concentration of morphine (0.57 μ g ml⁻¹) to the log₁₀ concentration of PGE₁ was -16.26 ± 3.71 , which was highly significant ($P < 0.005$).

In one experiment, 8 mM theophylline was added to the incubation mixture. In this experiment, stimulation by PGE₁ of cyclic AMP formation, which was 2.6 times that in the control, was within the usual range and morphine was effective in a dose-related way at the usual concentrations (0.57 and 5.7 μ g ml⁻¹).

Heroin and methadone likewise inhibited the stimulation by PGE₁ of cyclic AMP formation by rat brain homogenate. In the experiment of Fig. 3, the IC₅₀ values in μ g ml⁻¹ were: heroin, 0.159 (limits 0.037–0.314); methadone, 10.6 (limits 6.53–21.8). The slopes of the dose/response lines, derived as for morphine, with standard errors, were: heroin, 49.57 ± 9.957 ($P < 0.02$); methadone, 69.62 ± 7.38 ($P < 0.005$). Neither slope differed significantly from that of morphine in Fig. 1. In two experiments, dextrorphan, at 50 or 600 μ g ml⁻¹, was devoid of inhibitory activity.

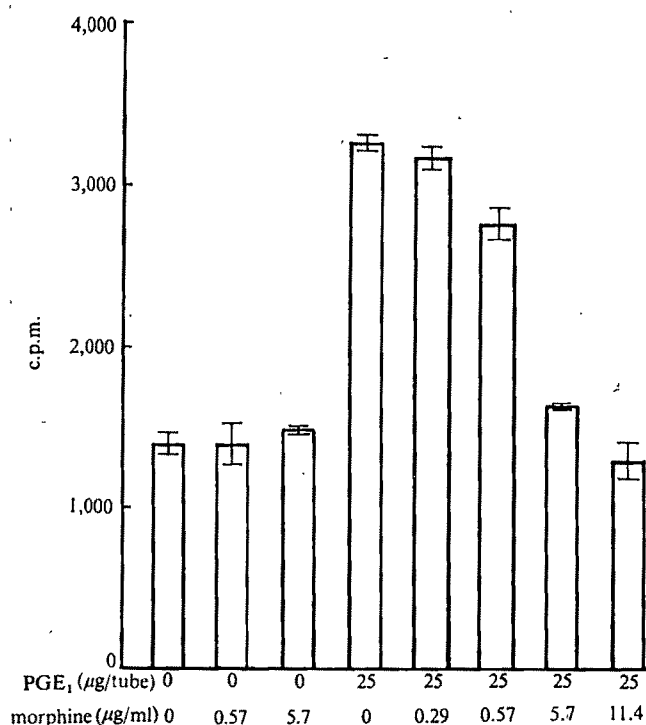


Fig. 1 Inhibition by morphine of the conversion of ³H-ATP to ³H-cyclic AMP stimulated by prostaglandin E₁ in rat brain homogenate. The amount of ³H-cyclic AMP formed after incubation for 20 min, in the presence or absence of 25 μ g per tube of PGE₁ and various concentrations of morphine, is expressed in mean c.p.m. \pm s.e.

We also tested whether naloxone antagonised the inhibitory effect of morphine on the stimulation by PGE₁ or PGE₂ of cyclic AMP formation. In both of two experiments, naloxone, at a molar ratio to morphine of 1:2, completely antagonised the effect of morphine. A feature of these experiments that will require further investigation was that naloxone alone also affected cyclic AMP formation.

In two preliminary experiments, using an analogous method, morphine also inhibited the stimulation by PGE₁ of cyclic AMP formation by homogenate of rat intestine. The IC₅₀ was roughly 2 μ g ml⁻¹.

Biochemical action and pharmacological effects

The stimulation by PGE₁ or PGE₂ of cyclic AMP formation by rat brain homogenate was probably due to the activation of an adenylyl cyclase; but, conceivably, it could have been due to inhibition of phosphodiesterase or of ATPase^{24,25}. That this effect of PGE₁ occurred in the presence of theophylline seems to rule out phosphodiesterase inhibition. Although the possibility that the effect was due to inhibition of ATPase seems slight and the activation of adenylyl cyclase by E prostaglandins is well documented, we prefer at this stage to speak of the stimulation of cyclic AMP formation rather than of adenylyl cyclase.

There seems little doubt that the interaction of E prostaglandins with the cyclic AMP system is a step in their mechanism of action in some cells. The above observations therefore raise the question: would inhibition of the stimulation by E prostaglandin of cyclic AMP formation in appropriate cells explain the mechanism of analgesic or other action of opiates? There are four lines of evidence bearing upon this question.

One line of evidence concerns the question of whether the occurrence and actions of E prostaglandins are such that their inhibition might explain some of the pharmacological effects of morphine. One such action of E prostaglandins is produc-

tion of pain or hyperalgesia; but this has so far been clearly demonstrated only in peripheral sites¹⁻³, whereas morphine is believed to act centrally²⁶⁻²⁸. There is some evidence, however, that in appropriate regions of the brain, E prostaglandins may also elicit pain or hyperalgesia. Thus, fever in which E prostaglandin is produced in the hypothalamus⁹, may be accompanied by severe headache or malaise. Again, in the rat, sodium salicylate, which inhibits E prostaglandin synthesis²⁰, lessens nociception generated by stimulation of the lateral hypothalamus with implanted electrodes²⁹. The mechanism whereby E prostaglandins might elicit or enhance pain centrally is uncertain; but they antagonise release or action of noradrenaline³⁰⁻³², which may exert an analgesic action³³⁻³⁵, and they may participate in acetylcholine release from nerve terminals¹⁷ and in conduction between neurones³⁶.

Because PGE₁ is thought to be "an ideal general mediator for raising body temperature"¹⁰, morphine might be expected to lower body temperature when this was being raised by production of E prostaglandins in the thermo-regulatory centres of the anterior hypothalamus. Morphine seems seldom to have been critically tested as an antipyretic while this mechanism was active; but we have traced one report in which this may have been done. In rats kept in a cold environment (5° C), injection of morphine, either 50 µg into the anterior hypothalamus or 50 mg kg⁻¹ intraperitoneally, did indeed lower body temperature, by a mean of 5-6° C (ref. 37). Whereas, when rats were maintained at 32° C, and PGE production in the thermoregulatory centres was presumably in abeyance, morphine by either route raised mean body temperature, by 1-2° C. In a thermoneutral environment (24° C) morphine had little effect in these experiments; but other investigators have found it to be mainly hypothermic when injected intracerebrally or systemically in the rat¹³. Tolerance can develop quickly to this hypothermic effect of morphine, revealing a hyperthermic component.

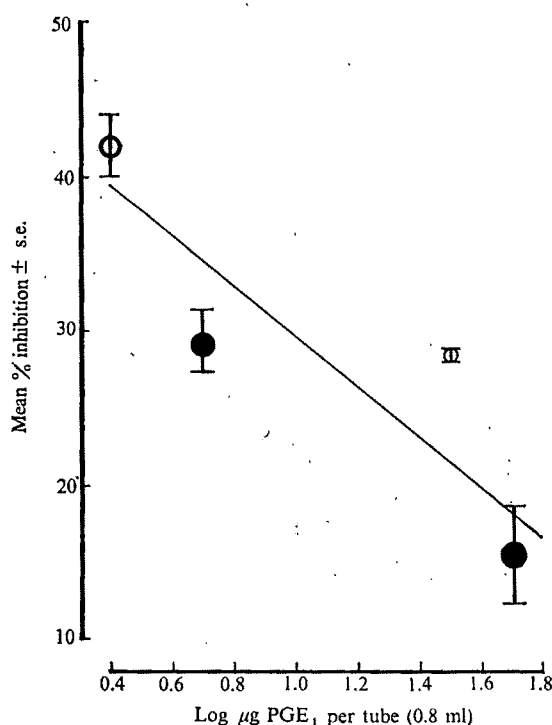


FIG. 2 Increased inhibition by morphine of the stimulation of cyclic AMP formation when the concentration of PGE₁ was reduced tenfold. The inhibitory effects in two experiments of one concentration of morphine (0.57 µg ml⁻¹), on the response to PGE₁ of rat brain homogenate are plotted against log concentration of prostaglandin.

Blockade of E prostaglandin in intestinal tissue might suppress diarrhoea, because E prostaglandin, which seems, with cyclic AMP to be involved in diarrhoea induced by ionising radiation³⁸ or toxins^{39,40}, increased fluid accumulation in the intestinal lumen^{4,5}.

A second line of evidence concerns antagonism between morphine, on the one hand, and cyclic AMP or PGE, on the other. If morphine acted as an analgesic by inhibiting PGE-activated cyclic AMP formation, we might expect that its potency would be reduced by administration of cyclic AMP. That cyclic AMP does indeed lessen the antinociceptive effect of morphine in the mouse and rat has been demonstrated⁴¹. There is evidence also that morphine can antagonise some effects of E prostaglandins on intestinal muscle¹⁵⁻¹⁷ and on the hypothalamus^{18,19}; and it has recently been proposed that morphine antagonises the mediation by E prostaglandins of acetylcholine release from guinea pig ileum¹⁷.

A third line of evidence is provided by our finding that the effectiveness of heroin, morphine and methadone, the ineffectiveness of the inactive stereoisomer, dextrorphan, and the antagonism of morphine by naloxone form a pattern consistent with the known pharmacological relationships of these drugs. Why the relative potencies of heroin, morphine and methadone against stimulation by PGE₁ of cyclic AMP formation slightly differ from their relative antinociceptive potencies *in vivo* could be explained in terms of the absorption and metabolism of these drugs, as discussed below.

Fourth, there is the question of how far the concentrations of opiates that inhibit the PGE stimulation of cyclic AMP formation *in vitro* correspond with those needed in tissues for antinociceptive effects *in vivo*. The antinociceptive ED₅₀ values of morphine obtained in the rat were 0.87 mg kg⁻¹ subcutaneously in the abdominal constriction test and 6.08 mg kg⁻¹ subcutaneously in the tail-pressure test⁴². After 5 mg kg⁻¹ morphine subcutaneously, values of 0.25 µg g⁻¹ and of 0.179 µg g⁻¹ were obtained for whole brain and 0.58 µg g⁻¹ for the hypothalamic area^{43,44}. In the experiment in Fig. 1, the IC₅₀ value of morphine for inhibiting the stimulation by PGE₁ of cyclic AMP formation in brain homogenate was 1.46 µg ml⁻¹. This, therefore, does not much exceed the value obtained for the hypothalamic concentration *in vivo* after a dose of the approximate ED₅₀ of morphine in the tail-pressure test. That morphine was more effective *in vitro* when less PGE₁ was used to stimulate cyclic AMP formation allows flexibility in matching *in vitro* and *in vivo* concentrations. Further flexibility could arise from the possibility that the drugs used might undergo some metabolism by rat brain homogenate *in vitro*.

Heroin is about three times as potent as morphine in the abdominal constriction test in the mouse⁴⁵, but its true analgesic potency is masked by its rapid conversion in the body to morphine and 6-monoacetylmorphine⁴⁶. In the guinea pig isolated ileum, heroin was about twice as active as morphine in a test that correlates well with analgesic activity⁴⁷. In antinociceptive tests in mouse, rat and rabbit, methadone is slightly more potent than morphine, but methadone is much better absorbed into the brain⁴⁸. In the rabbit, for example, to provide an equal antinociceptive effect, a concentration of methadone approximately thirty times higher than that of morphine is needed in the brain²⁸. The concentrations of heroin, morphine and methadone that inhibit the stimulation by PGE₁ of cyclic AMP formation *in vitro* thus probably correspond with the concentrations of drug or active derivative that are effective *in vivo*. Moreover, the relative potencies of (—) morphine and (±) methadone and the ineffectiveness of dextrorphan are consistent with their affinities for the opiate receptor of rat brain homogenate⁴⁸.

Thus, four lines of evidence support this analysis of opiate action. First, some actions of E prostaglandins are such that their antagonism would explain some of the main

pharmacological effects of opiates. Second, cyclic AMP antagonises the antinociceptive effect of morphine and morphine antagonises some effects of E prostaglandins. Third, the relationship between heroin, morphine, methadone, dextrorphan and naloxone in our experiments *in vitro* largely corresponds with their pharmacological relationship

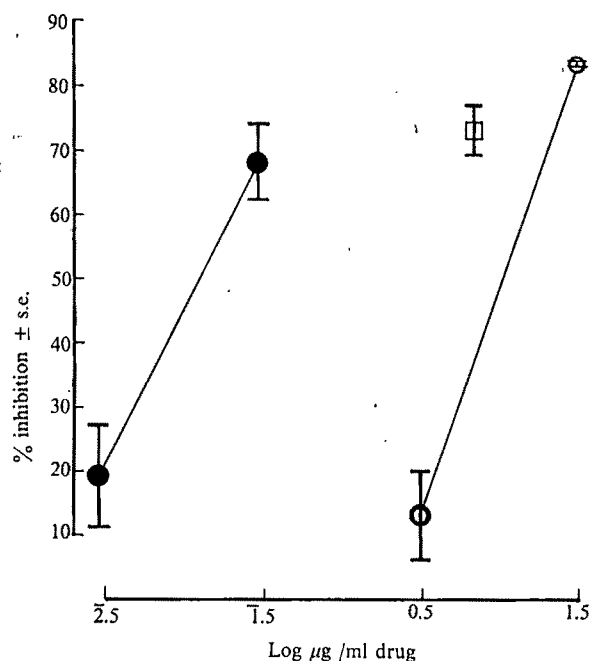


FIG. 3 Inhibition by heroin (●), morphine (□) and methadone (○) of the stimulation by PGE_1 of cyclic AMP formation in rat brain homogenate.

in vivo. Fourth, the concentrations at which heroin, morphine and methadone inhibit PGE_1 *in vitro* roughly correspond with effective concentrations in brain *in vivo*. We therefore propose that the ability of opiates to inhibit the stimulation by E prostaglandins of cyclic AMP formation in rat brain homogenate, presumably by inhibiting stimulation of a neuronal adenylyl cyclase, represents a biochemical mechanism that could account for the analgesic and allied effects of these drugs. Such a mechanism could accommodate interactions of opiates with other humoral messengers: If this is how opiates act, then tolerance and dependence, which arise from the agonist action of opiates⁴⁹, are enhanced by cyclic AMP⁵⁰ and are partly blocked by indomethacin⁵¹, may represent a compensating hypertrophy of a part of the inhibited PGE-cyclic AMP mechanism.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Possible observation of tachyons associated with extensive air showers

SEVERAL searches¹⁻⁴ have been made for tachyons using either laboratory particle sources or high energy cosmic rays. Effects associated with their supposed characteristic mass and velocity have been searched for but so far no positive evidence has been reported. As has been repeatedly pointed out, however, the goal of these searches is so important that all possible avenues should be fully investigated. We report here apparently positive results from a pilot search for tachyons associated with cosmic ray showers of energy about 2×10^{15} eV.

The first interaction of a primary cosmic-ray nucleon occurs at a typical altitude of 20 km (ref. 5). Further interactions result in a cascade of relativistic particles travelling with speeds close to that of light (c). Thus, most of the particles in this extensive air shower (EAS) arrive at sea level with a time spread of only a few nanoseconds⁶. If any shower particles are produced with velocities greater than c , they should be observable in the time interval up to 20 km/ c (60 μ s) before the arrival of the shower front. The precise time depends on their velocity and production altitude. A pilot experiment has been conducted to search for particles arriving within this time period. In this experiment, the EAS which were studied had energies some two orders of magnitude higher than in previously reported work⁴.

A plastic scintillator was used to detect the particles. It is not clear what interactions a tachyon might have with the atmosphere or the scintillator. The present search was made assuming that tachyons are produced in EAS interactions at heights between 20 km and 400 m (800 kg m⁻² and 10,000 kg m⁻²) and have a sufficiently long absorption length for some to reach the detector. Detection might be accomplished by direct interaction of the tachyons with the scintillator or through the production of secondary particles which interact with the detector. It is not necessary that a single tachyon should produce a large response in the scintillator (as, for instance, a charged relativistic particle would). In principle, provided that observations are initiated by the detection of EAS, it is possible to sum results from many observations so that small non-random effects can be observed.

The chief experimental difficulty with this procedure is that if a recording device is triggered by the arrival of an EAS, it will be too late to observe the tachyon unless substantial signal delays are inserted. This was overcome in the present case by the use of a digital transient recorder (Biomation International, Palo Alto, California; model 610B) which enabled us to trigger our recording system from an EAS and then examine the signal from the particle detector recorded prior to the arrival of the trigger. The device continually samples and digitises (to six bit accuracy) the output of the particle detector. Two hundred and fifty-six words of this digitised data are stored in a shift register which is continually updated. Thus, when a trigger is received, the previous 256 words of data are in store (in our case representing 128 μ s) and can be output at leisure. Output was to a chart recorder after digital to analogue conversion. In this mode, the recorder only outputs 228 words (114 μ s).

The EAS trigger was obtained from the fast timing part of the air shower array at the Buckland Park field station of the University of Adelaide. Five 1 m square plastic scintillators, 50 mm thick, were used, in a square array of side 30 m, one scintillator being at each corner with one at the centre. Each scintillator was viewed with a Philips XP1040 photomultiplier and the arrival of an EAS was detected by a coincidence between the centre detector and any three of the outer detectors with a resolving time of 150 ns. Thus, air showers were detected from a cone about the zenith, with half angle of about 35°. The mean rate of showers was one per 500 s, corresponding to a minimum shower energy of about 2×10^{15} eV. In addition, one of the corner scintillators was also viewed by an RCA 8055 photomultiplier connected to a charge sensitive preamplifier, the output of which was the signal recorded by the transient recorder. The impulse response of the system had a width of 1.7 μ s at half maximum ensuring that a sampling interval of 0.5 μ s gave an acceptable reconstruction of the waveform on digital to analogue conversion.

Data from a total of 1,307 air showers, detected between February and August, 1973, have been analysed. The aim of the analysis was to demonstrate whether or not non-random effects were observable immediately preceding an EAS and for this reason a simple analysis procedure was employed. The time (with respect to the arrival of each EAS) of the largest excursion of the amplified photomultiplier output was noted. If there was doubt as to which of a number of pulses was the largest, all the apparently equal pulses were included. In practice, approximately 4% of the events had their two largest pulses sufficiently close in amplitude to cause ambiguity. In order to check on observer bias in assigning relative pulse heights, approximately 600 events were re-read by an independent chart reader. Some 3% of the events had the assignment of the largest pulse changed but no systematic bias was found.

The position of the shower front on the output trace can be adjusted; thus the time interval available for analysis is dependent on the exact setting of the transient recorder. For 1,176 of the events the record extended beyond 105 μ s before the air shower arrival. Figure 1a shows a histogram of the positions of the largest pulses for these events. The figure suggests that the distribution of the largest pulses is not uniform and a χ^2 test on the data indicates that there is only 1% chance that the data is from a uniform distribution.

In order to include data from all 1,307 recorded events it is necessary to limit the period of analysis to 97.5 μ s before the shower arrival. The resulting histogram is shown in Fig. 1b. A χ^2 test on this data shows less than 0.1% probability that the data is from a uniform distribution. In addition, since if tachyons are observed one might expect their arrival times to be spread over more than one 7.5 μ s bin, one can also test to see whether there are specific time regions contributing excessively to the non uniformity or whether the large χ^2 is due to a more or less random time distribution of excesses. This problem has been discussed by David⁷ who noted that a calculation of χ^2 involved the squaring of deviations from a hypothetical set and that independent information on the sign of the deviations was ignored. An examination of the distribution of bins above and below the mean shows that the probability that our data are selected from a uniform distribution is less than 1%, on the basis of a non-parametric run test.

The data were obtained in 12 runs containing between 70 and 180 events each and the data were tested for the possibility of non-random variations between runs. This was done

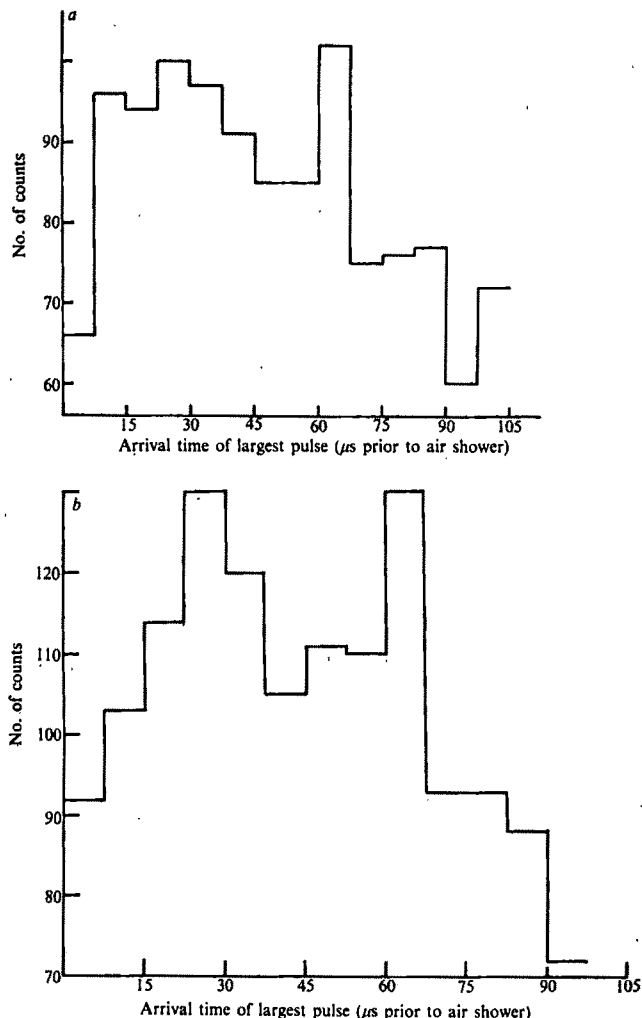


FIG. 1 The time distribution of largest photomultiplier pulse in the period prior to the arrival of an extensive air shower; *a*, data from 1,176 air showers in 14 bins of width 7.5 μ s; *b*, data from all 1,307 showers observed in 13 bins of width 7.5 μ s.

by comparing data obtained from individual runs with the final distribution. Taking deviations of each bin of each run from the normalised final distribution gave a χ^2 indicating less than a 10% probability that the individual runs were not

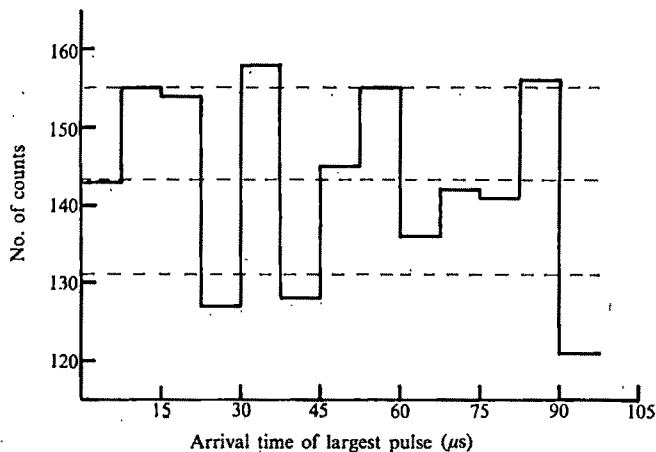


FIG. 2 The time distribution of largest photomultiplier pulses in 1,839 random samples of output in 13 bins of width 7.5 μ s.

randomly selected subsets of the total data.

The experiment has the unique quality that spurious pickup in the electronics is most unlikely to affect the result since pickup must occur at or after the arrival of the shower front, thus arriving after recording and storage of the data. It is arguable that the arrival of an air shower could generate interference in the recorder memory. If this were so, unless

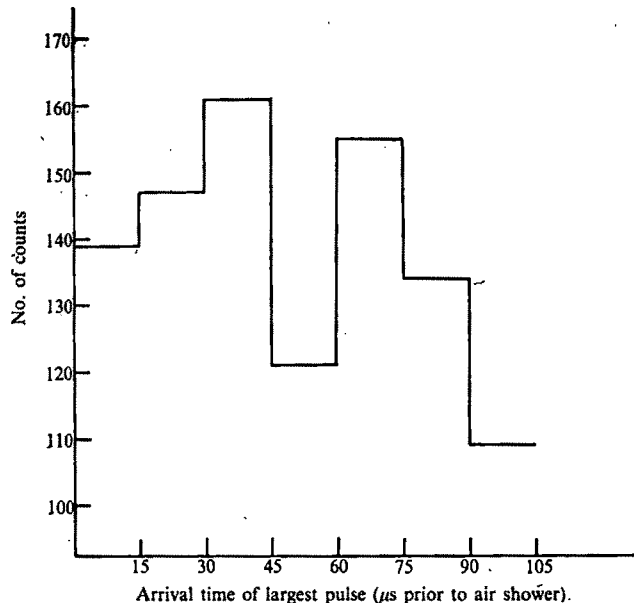


FIG. 3 The time distribution of largest photomultiplier pulses in the 105 μ s period prior to 978 extensive air showers. The data was recorded at half the sampling rate of that in Figs 1 and 2 and analysed into 7 bins of width 15 μ s.

only the least significant bit were affected, a discontinuity in the trace would be expected after digital to analogue conversion. In more than 3,000 events no such effect was observed. In addition, grossly overloading the input amplifier with a pulse was found to have no effect on stored data.

In case there was some other form of pickup or of observer bias, we have triggered our coincidence system with artificial pulses and repeated the analysis on an apparently random sample of photomultiplier output; 1839 events were analysed in the same way as the air shower triggered data indicating on the basis of a χ^2 test a probability of about 40% that these test results were from a uniform distribution. The data are presented in Fig. 2. The probability that this test data and the EAS data are from the same distribution is less than one in 10^4 on the basis of a χ^2 test.

We have also operated our transient recorder at one half the previous sampling rate (with a lower bandwidth) and taken results over a similar range to that described above. These data from a further 972 showers were analysed into seven coarser bins (15 μ s wide) but otherwise a similar analysis technique was employed. The resulting distribution shown in Fig. 3 seems to exhibit the same broad features as Fig. 1. We use this as further evidence that the equipment and analysis technique are largely free from bias since the equipment was now operated in a rather different manner.

Ramana Murthy⁴ has reported an unsuccessful search for tachyons in EAS. Two detection techniques were employed, in one of which a liquid scintillator was used and a search made for particles immediately prior to EAS. This experiment is rather similar to the one described above but differs in a number of important respects. In order to avoid the use of long analogue delays, his measurements were initiated by the detection of single charged particles and a search was made for EAS following within 19.2 μ s. This technique is much less efficient than ours since only 1 in 250 potential tachyons was followed by an EAS within the time of interest. Also, potential tachyons have the additional constraint that

they must produce a response in the scintillator comparable with that produced by a conventional charged particle. As mentioned earlier, the EAS studied by Ramana Murthy were almost two orders of magnitude less energetic than those used by us and also a much smaller time range was examined. Since the peak in our distribution occurs after the 18 μ s time interval investigated by Ramana Murthy, the results cannot be directly compared. But a statistical examination of his published data using David's technique indicates that there is less than 5% probability that the data is from a uniform distribution. We note that, subjectively, the observed distribution seems to rise in the period prior to 13 μ s before the shower arrival. This is not inconsistent with our observations.

It is possible that an explanation may be found for these results without invoking the existence of tachyons. A. G. Gregory has pointed out to us that fission or spallation in the interstellar medium or production of associated particles at the source might account for the correlated arrival of cosmic rays. We note, however, that unless particles are produced with closely similar rigidity and velocity vectors they are unlikely to remain associated for long in the interstellar or source magnetic fields. A closely related problem has been discussed by Weekes⁸ in connection with pulsar periodicities in cosmic-ray arrival times.

We conclude that we have observed non-random events preceding the arrival of an extensive air shower. Being unable to explain this result in a more conventional manner, we suggest that this is the result of a particle travelling with an apparent velocity greater than that of light.

We thank Dr A. G. Gregory and Professors C. A. Hurst and J. R. Prescott for comments, Mr K. W. Morris for advice on statistical analysis, and Ms J. M. Taylor for chart reading. One of us (R.W.C.) holds a Queen Elizabeth Fellowship. The University of Calgary is thanked for the loan of equipment.

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Black hole explosions?

QUANTUM gravitational effects are usually ignored in calculations of the formation and evolution of black holes. The justification for this is that the radius of curvature of space-time outside the event horizon is very large compared to the Planck length $(G\hbar/c^3)^{1/2} \approx 10^{-33}$ cm, the length scale on which quantum fluctuations of the metric are expected to be of order unity. This means that the energy density of particles created by the gravitational field is small compared to the space-time curvature. Even though quantum effects may be small locally, they may still, however, add up to produce a significant effect over the lifetime of the Universe $\approx 10^{17}$ s which is very long compared to the Planck time $\approx 10^{-43}$ s.

The purpose of this letter is to show that this indeed may be the case; it seems that any black hole will create and emit particles such as neutrinos or photons at just the rate that one would expect if the black hole was a body with a temperature of $(\kappa/2\pi)(\hbar/2k) \approx 10^{-6} (M_\odot/M)K$ where κ is the surface gravity of the black hole¹. As a black hole emits this thermal radiation one would expect it to lose mass. This in turn would increase the surface gravity and so increase the rate of emission. The black hole would therefore have a finite life of the order of $10^{71} (M_\odot/M)^{-3}$ s. For a black hole of solar mass this is much longer than the age of the Universe. There might, however, be much smaller black holes which were formed by fluctuations in the early Universe². Any such black hole of mass less than 10^{15} g would have evaporated by now. Near the end of its life the rate of emission would be very high and about 10^{30} erg would be released in the last 0.1 s. This is a fairly small explosion by astronomical standards but it is equivalent to about 1 million 1 Mton hydrogen bombs.

To see how this thermal emission arises, consider (for simplicity) a massless Hermitean scalar field ϕ which obeys the covariant wave equation $\phi_{;ab}g^{ab} = 0$ in an asymptotically flat space time containing a star which collapses to produce a black hole. The Heisenberg operator ϕ can be expressed as

$$\phi = \sum_i \{f_i a_i + \bar{f}_i a_i^+\}$$

where the f_i are a complete orthonormal family of complex valued solutions of the wave equation $f_{i;ab}g^{ab} = 0$ which are asymptotically ingoing and positive frequency—they contain only positive frequencies on past null infinity I^- ^{3,4,5}. The position-independent operators a_i and a_i^+ are interpreted as annihilation and creation operators respectively for incoming scalar particles. Thus the initial vacuum state, the state containing no incoming scalar particles, is defined by $a_i|0\rangle = 0$ for all i . The operator ϕ can also be expressed in terms of solutions which represent outgoing waves and waves crossing the event horizon:

$$\phi = \sum_i \{p_i b_i + \bar{p}_i b_i^+ + q_i c_i + \bar{q}_i c_i^+\}$$

where the p_i are solutions of the wave equation which are zero on the event horizon and are asymptotically outgoing, positive frequency waves (positive frequency on future null infinity I^+) and the q_i are solutions which contain no outgoing component (they are zero on I^+). For the present purposes it is not necessary that the q_i are positive frequency on the horizon even if that could be defined. Because fields of zero rest mass are completely determined by their values on I^- , the p_i and the q_i can be expressed as linear combinations of the f_i and the \bar{f}_i :

$$p_i = \sum_j \{\alpha_{ij} f_j + \beta_{ij} \bar{f}_j\} \quad \text{and so on}$$

The β_{ij} will not be zero because the time dependence of the metric during the collapse will cause a certain amount of mixing of positive and negative frequencies. Equating the two expressions for ϕ , one finds that the b_i , which are the annihilation operators for outgoing scalar particles, can be expressed as a linear combination of the ingoing annihilation and creation operators a_i and a_i^+

$$b_i = \sum_j \{\bar{\alpha}_{ij} a_j - \bar{\beta}_{ij} a_j^+\}$$

Thus when there are no incoming particles the expectation value of the number operator $b_i^+ b_i$ of the i th outgoing state is

$$\langle 0_- | b_i^+ b_i | 0_- \rangle = \sum_j |\beta_{ij}|^2$$

The number of particles created and emitted to infinity in a gravitational collapse can therefore be determined by calculating the coefficients β_{ij} . Consider a simple example in which

the collapse is spherically symmetric. The angular dependence of the solution of the wave equation can then be expressed in terms of the spherical harmonics Y_{lm} and the dependence on retarded or advanced time u, v can be taken to have the form $\omega^{-1/2} \exp(i\omega u)$ (here the continuum normalisation is used). Outgoing solutions $p_{lm\omega}$ will now be expressed as an integral over incoming fields with the same l and m :

$$p_{\omega} = \int \{\alpha_{\omega\omega'} f_{\omega'} + \beta_{\omega\omega'} \bar{f}_{\omega'}\} d\omega'$$

(The lm suffixes have been dropped.) To calculate $\alpha_{\omega\omega'}$ and $\beta_{\omega\omega'}$ consider a wave which has a positive frequency ω on I^+ propagating backwards through spacetime with nothing crossing the event horizon. Part of this wave will be scattered by the curvature of the static Schwarzschild solution outside the black hole and will end up on I^- with the same frequency ω . This will give a $\delta(\omega - \omega')$ behaviour in $\alpha_{\omega\omega'}$. Another part of the wave will propagate backwards into the star, through the origin and out again onto I^- . These waves will have a very large blue shift and will reach I^- with asymptotic form

$$C\omega^{-1/2} \exp\{-i\omega\kappa^{-1} \log(v_0 - v) + i\omega v\} \text{ for } v < v_0$$

and zero for $v \geq v_0$, where v_0 is the last advanced time at which a particle can leave I^+ , pass through the origin and escape to I^+ . Taking Fourier transforms, one finds that for large ω' , $\alpha_{\omega\omega'}$ and $\beta_{\omega\omega'}$ have the form:

$$\begin{aligned} \alpha_{\omega\omega'} &\approx C \exp[i(\omega - \omega')v_0](\omega'/\omega)^{1/2} \\ &\quad \cdot \Gamma(1 - i\omega/\kappa)[-i(\omega - \omega')]^{-1+i\omega/\kappa} \\ \beta_{\omega\omega'} &\approx C \exp[i(\omega + \omega')v_0](\omega'/\omega)^{1/2} \\ &\quad \cdot \Gamma(1 - i\omega/\kappa)[-i(\omega + \omega')]^{-1+i\omega/\kappa} \end{aligned}$$

The total number of outgoing particles created in the frequency range $\omega \rightarrow \omega + d\omega$ is $d\omega \int_{\omega'} |\beta_{\omega\omega'}|^2 d\omega'$. From the above expression it can be seen that this is infinite. By considering outgoing wave packets which are peaked at a frequency ω and at late retarded times one can see that this infinite number of particles corresponds to a steady rate of emission at late retarded times. One can estimate this rate in the following way. The part of the wave from I^+ which enters the star at late retarded times is almost the same as the part that would have crossed the past event horizon of the Schwarzschild solution had it existed. The probability flux in a wave packet peaked at ω is roughly proportional to $\int_{\omega_1}^{\omega_2} \{|\alpha_{\omega\omega'}|^2 - |\beta_{\omega\omega'}|^2\} d\omega'$ where $\omega_2 \gg \omega_1 \gg 0$. In the expressions given above for $\alpha_{\omega\omega'}$ and $\beta_{\omega\omega'}$ there is a logarithmic singularity in the factors $[-i(\omega - \omega')]^{-1+i\omega/\kappa}$ and $[-i(\omega + \omega')]^{-1+i\omega/\kappa}$. Value of the expressions on different sheets differ by factors of $\exp(2\pi n\omega/\kappa)$. To obtain the correct ratio of $\alpha_{\omega\omega'}$ to $\beta_{\omega\omega'}$ one has to continue $[-i(\omega + \omega')]^{-1+i\omega/\kappa}$ in the upper half ω' plane round the singularity and then replace ω' by $-\omega'$. This means that, for large ω' ,

$$|\alpha_{\omega\omega'}| = \exp(\pi\omega/\kappa) |\beta_{\omega\omega'}|$$

From this it follows that the number of particles emitted in this wave packet mode is $(\exp(2\pi\omega/\kappa) - 1)^{-1}$ times the number of particles that would have been absorbed from a similar wave packet incident on the black hole from I^- . But this is just the relation between absorption and emission cross sections that one would expect from a body with a temperature in geometric units of $\kappa/2\pi$. Similar results hold for massless fields of any integer spin. For half integer spin one again gets a similar result except that the emission cross section is $(\exp(2\pi\omega/\kappa) + 1)^{-1}$ times the absorption cross section as one would expect for thermal emission of fermions. These results do not seem to depend on the assumption of exact spherical symmetry which merely simplifies the calculation.

Beckenstein⁶ suggested on thermodynamic grounds that some multiple of κ should be regarded as the temperature of a black hole. He did not, however, suggest that a black hole could emit particles as well as absorb them. For this reason Bardeen, Carter and I considered that the thermodynamical similarity between κ and temperature was only an analogy. The present result seems to indicate, however, that there may be more to it than this. Of course this calculation ignores the back reaction of the particles on the metric; and quantum fluctuations on the metric. These might alter the picture.

Further details of this work will be published elsewhere. The author is very grateful to G. W. Gibbons for discussions and help.

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Absorption and emission by interstellar CH at 9 cm

RYDBECK, Ellender and Irvine¹ have recently detected the 9-cm lines of the $^2\Pi_{1/2}$, $J = 1/2$ doublet of interstellar CH. The $F = 1 \rightarrow 1$ transition at 3,335.475 MHz was observed in emission in a wide range of galactic sources ranging from dark clouds to the spiral arms in front of Cassiopea A. The two satellite transitions $F = 0 \rightarrow 1$ (at 3,263.788 MHz) and $F = 1 \rightarrow 0$ (at 3,349.185 MHz) were also observed in emission in several sources.

We have observed the 3,335.475 MHz transition of CH in several southern galactic sources. In RCW38 this line is seen in absorption, while the two satellite lines are seen in emission. In several sources the distribution of CH is found to be extended.

The observations were made on December 10 and 11, 1973, with the Parkes 64-m telescope equipped with a 9-cm parametric amplifier having a noise temperature of 150 K. The telescope beam at 9 cm is 6 arc min. The receiver output was analysed by a 512-channel digital correlator producing a spectral resolution of 19.5 kHz.

The Onsala observations¹ of CH emission at 3,335.475 MHz from Cloud 2 and W12 were confirmed. In Cloud 2 we measured an antenna temperature similar to that found with the Onsala 25-m telescope. For W12 it was 0.23 K, about 50% greater than the Onsala value of 0.15 K; however, at a position 5 arc min south (where the continuum intensity had fallen to one seventh of its peak value) the line signal had decreased by only 30%. A similar situation occurred in RCW36. Thus the CH distribution is considerably more extended than the continuum for these HII regions; in Cloud 2 it must be comparable with the 16 arc min beam of the Onsala 25-m telescope.

In RCW38 the 3,335.475 MHz CH line was observed in absorption (Fig. 1); this is the first case found of absorption by CH. But the two satellite lines at 3,263.788 and 3,349.185 MHz appear in emission, with an antenna temperature of 1.3 K for the former. The pattern of main line absorption and satellite line emission indicates substantial departures from thermodynamic equilibrium in the population distribution.

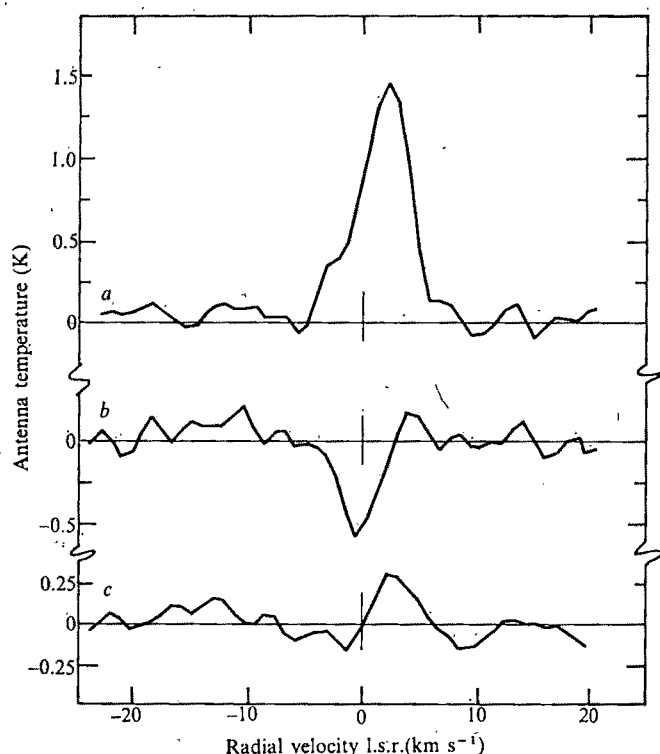


Fig. 1 9-cm lines of the $^2\Pi_{1/2}$, $J = 1/2$, CH Λ doublet observed in RCW38. Channel spacing is 10 kHz \equiv 2.1 km s $^{-1}$. The local standard of rest radial velocities are based on the rest frequencies determined by Rydbeck *et al.*¹, a, $F = 0 \rightarrow 1$ at 3,263.788 \pm 0.010 MHz; b, $F = 1 \rightarrow 1$ at 3,335.475 \pm 0.010 MHz; c, $F = 1 \rightarrow 0$ at 3,349.185 \pm 0.010 MHz.

A comparison of the CH profiles for RCW38 in Fig. 1 with the 18-cm OH profiles in Fig. 2 (from ref. 2) shows that the CH and OH profiles must contain at least two velocity components. The higher velocity component appears in absorption in OH at 1,665, 1,667 and 1,720 MHz, and in emission in OH at 1,612 MHz, and in CH at 3,264 and 3,349 MHz (and perhaps at 3,335 MHz). The lower velocity component appears in absorption on all four OH lines and in CH at 3,335 MHz, while it is in emission in CH at 3,264 MHz. Detailed comparison of the CH and OH profiles is restricted by the present limited accuracy of the rest frequencies deduced by Rydbeck *et al.*¹ (\pm 10 kHz, equivalent to \pm 1 km s $^{-1}$ in radial velocity).

The CH population distribution in RCW38 required to explain the substantial departures from LTE could be similar to that of the $^2\Pi_{1/2}$, $J = 1/2$ levels of OH in Sagittarius B2 advanced by Gardner and Ribes.³ With their model, maser amplification of the $F = 0 \rightarrow 1$ transition occurs; as it is the region in front of the continuum source which is sampled we would expect the CH emission profile to resemble that for the OH absorption, as it does; we also find that the $F = 0 \rightarrow 1$ line intensity decreases in proportion to the continuum when the beam is pointed 6 arc min north of the source maximum. The model indicates that there is no inversion of the $F = 1 \rightarrow 1$ transition; since we observe an absorption

line the excitation temperature for this transition must be less than the peak brightness temperature in RCW38, which is about 1,500 K (from the emission measure of ref. 4). For the $F = 1 \rightarrow 0$ transition there might not be inversion,

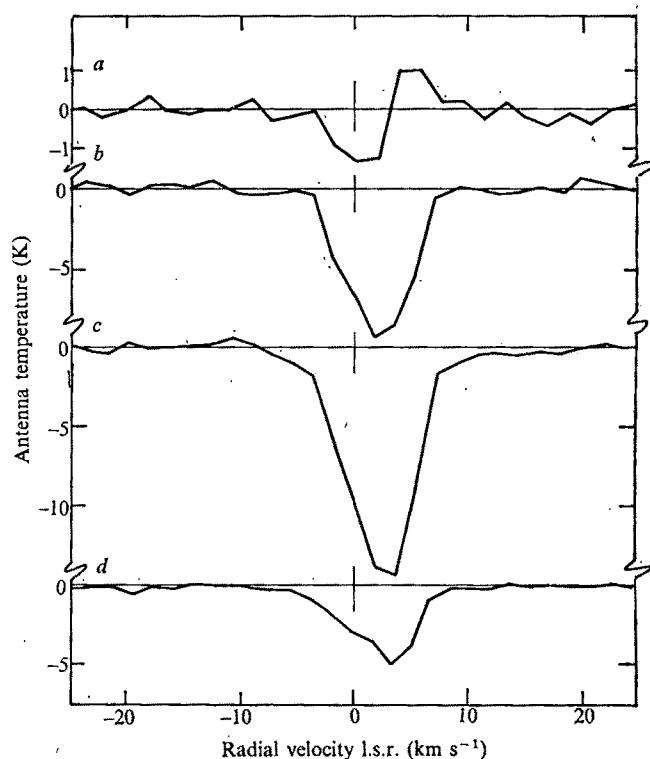


Fig. 2 18-cm lines of the $^2\Pi_{3/2}$, $J = 3/2$ OH Λ doublet observed in RCW38 (from Manchester *et al.*²). Filter bandwidth is 10 kHz \equiv 1.8 km s $^{-1}$. a, 1,612 MHz; b, 1,665 MHz; c, 1,667 MHz; d, 1,720 MHz.

although the excitation temperature is probably higher than for $F = 1 \rightarrow 1$ since the line is observed in emission. Such a population distribution could result from a process of formation or excitation which preferentially filled the upper $F = 0$ level.

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Observations of Sgr A at 160 MHz with 1.9 arc min resolution

THE radio source Sgr A, which is located at or near the galactic centre, has now been observed with high resolution (<1 arc min) at several frequencies between 327 and 15 GHz¹⁻⁴. Downes and Martin³ have suggested that two main components are present between 1,400 and 5,000 MHz: Sgr A-West, identified with the galactic nucleus, coincides with the position of the $2.2\ \mu\text{m}$ source of Becklin and Neugebauer⁵. Its size is about 45 arc s and it may contain smaller scale structure^{3,4}. Sgr A-East, about 1.5 arc min to the east, is larger (~ 2.5 arc min) and has a steeper spectrum³ so that it dominates at low frequencies.

Here we extend the high resolution observations of Sgr A down to 160 MHz. This is about the lowest frequency at which observations are possible because the exponential fall-off in flux makes the source undetectable below ~ 120 MHz.

The 160 MHz observations were made with the radio-heliograph at Culgoora, NSW^{6,7}. This instrument has a beam diameter at the zenith of 1.9 arc min (Sgr A passes within 1 arc deg of the zenith). Each observation consisted of a series of about 10 drift scans made with 16 heliograph beams which were arranged to straddle the declination of the source. The 10 drift scans were averaged and used to produce a map of the brightness distribution over an area of 30×17 arc min. Ten such maps, obtained on different occasions and at a variety of hour angles, were superimposed to produce the final map. This procedure minimises the effects due to noise and the presence of other sources in the grating lobes of the radioheliograph. The beam-forming procedure in the heliograph is a switched system in which the area of the sky within about 4 arc deg of the beam is used as a reference. Thus the heliograph does not respond to large scale galactic structure and the temperature of the base level underlying a discrete source is unknown.

Figure 1 shows the brightness distribution of Sgr A obtained by this procedure. There seems to be a small compact source, possibly unresolved, which is apparently superimposed on an extended component about 8 arc min in diameter. The position of the peak, RA 17 h 42 min 33.6 ± 1.0 s, dec. $-28^\circ 58' 40'' \pm 20''$ (1950), is almost exactly the same as that in Little's⁸ map at 408 MHz. This compact source probably corresponds to Sgr A-East.

Krishna *et al.*² have observed Sgr A at 327 MHz using lunar occultations which gave an effective resolution of $\lesssim 1$

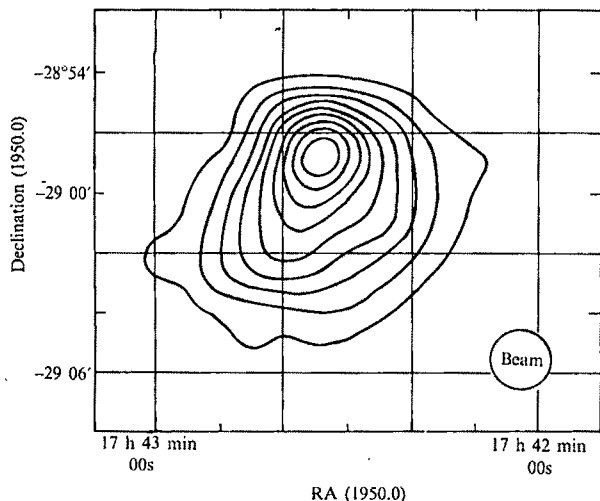


Fig. 1 Contour diagram of Sgr A at 160 MHz. The peak main beam brightness temperature, T_p , is 6.3×10^4 K. Contour levels are 10%, 20%, . . . 90% of T_p .

arc min. They claim to have detected Sgr A-West (they refer to it as component "A" but give no flux density) and have suggested that Sgr A-East contains a compact component ("S") which is slightly weaker than Sgr A-West and separated from it by about 1.5 arc min. We have examined each of our individual maps at 160 MHz (as well as the combined map of Fig. 1) and Little's map at 408 MHz (where the resolution is 2.9 arc min) and can find no indication of Sgr A-West or of any splitting into two subsources. If Sgr A-West is no stronger at 160 and 408 MHz than reported by Downes and Martin³ at 1,400, 2,700 and 5,000 MHz (30 f.u.), then we would not expect to see it. We conclude that the presence of Sgr A-West at frequencies lower than 1,400 MHz is not confirmed.

The extended component seen on Fig. 1 probably corresponds to the extended component (designated "E") of Krishna *et al.*², but its shape is considerably different. This component is also prominent on the 408 MHz map of Little⁸, where its shape is generally similar to that on Fig. 1, but the brightness falls off more slowly to the north and the ridge line to the south-east is less prominent than at 160 MHz, probably because of the somewhat lower resolution.

The measured flux density of Sgr A at 160 MHz is 114 ± 16 f.u. (16 f.u. is twice the expected error derived from 10 independent measurements.) This value includes the contributions of both the extended and compact components. On several occasions we have repeated Dulk's⁹ 80 MHz observations of Sgr A. We have made no consistent detections of the source and the upper limit of 5 f.u. remains unchanged.

Figure 2 shows most of the published measurements of the flux density of Sgr A. The measurements of the total flux density between about 400 MHz and 20 GHz can be fitted by a power-law spectrum with spectral index ~ -0.25 . As seen on Fig. 2, however, a more consistent interpretation is obtained if we assume that Sgr A-West has a nearly flat spectrum and the remainder (Sgr A-East) has a straight spectrum with index ~ -0.47 . Sgr A-West, with its small scale structure^{3,4}, could have a more complex spectrum than portrayed on Fig. 2 but no component could have a spectral index very much steeper than -0.5 .

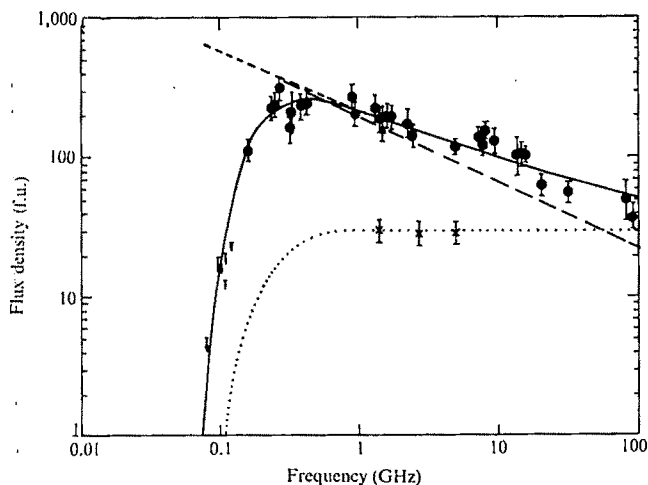


Fig. 2 Spectrum of Sgr A including the present value at 160 MHz. The dashed, dotted and full lines show the assumed spectra for Sgr A-East, Sgr A-West and the total flux density respectively. The falloff at low frequencies was derived from the extrapolated spectrum and the present measurement at 160 MHz. The points marked with crosses and triangles represent observations where Sgr A-East and Sgr A-West were resolved.

We now consider the decrease in flux at frequencies $\lesssim 400$ MHz. Neither a change in the electron energy spectrum nor a mixture of emission and absorption regions within the

source can account for the exponential decrease in flux. Instead it has been attributed to free-free absorption in the interstellar medium between Sgr A and the Earth^{9,10}. If we assume that the intrinsic spectrum of the non-thermal component continues unchanged to low frequencies, then the observed fall-off requires the optical depth at 160 MHz to be $\tau_{160} = 1.34$. The solid curve on Fig. 2 is the theoretical flux density curve which passes through our point at 160 MHz.

We can use the value of optical depth at 160 MHz to estimate the r.m.s. electron density $\langle n_e^2 \rangle^{1/2}$ in the interstellar medium if we make some assumptions about the electron temperature and filling factor. For a hot diffuse medium with $T_e = 5,000$ K distributed over the full 10 kpc to Sgr A we find $\langle n_e^2 \rangle^{1/2} \approx 1.6 \text{ cm}^{-3}$. For cold, dense clouds with $T_e \approx 50$ K occupying 2% of the line of sight we find $\langle n_e^2 \rangle^{1/2} \approx 0.6 \text{ cm}^{-3}$.

Lockman and Gordon¹¹ have observed weak recombination line emission at the position of Sgr A and at other locations within about 0.5 arc deg where no discrete source is visible. The range of T_e and n_e derived from these observations is in generally good agreement with our values above. In interpreting their observations, however, Lockman and Gordon¹¹ and Gordon⁴ have suggested an extremely cold, dense cloud model in which $n_e > 3 \text{ cm}^{-3}$ and $T_e \approx 20$ K. In such an extreme cloud, the absorption observed at 80 and 160 MHz would occur in a distance of only 3 pc. A cloud of this size, if located near the galactic centre, would subtend an angle of only 1 arc min. Our 80 and 160 MHz observations, however, suggest that the absorption is fairly uniform over an area of ≥ 50 (arc min)²; it is not patchy, as would be implied by this cloud model. We conclude that the extremely cold, dense cloud model is unlikely; to cover the source there must be one large cloud or many small clouds, each having a density lower than 3 cm^{-3} , a temperature higher than 20 K or both.

Observations of the radiation from supernova remnants show that absorption in the galactic plane is fairly common¹²⁻¹⁴, although not as strong as for Sgr A, and that the absorption is fairly smooth over the angular extent of the sources¹⁵.

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Upper limit to the flux of soft X rays from λ -Sco

DURING a rocket flight on May 26, 1971 which carried a number of large area thin window proportional counters, a soft X-ray source was observed by Bleeker *et al.*¹ in Scorpio. Photons in the energy range 0.37 keV to 1.9 keV were detected from a position error box of approximately 40 square degrees. This admittedly large error box contains the bright star λ -Sco and it was suggested that this object may be the source of the soft X-ray emission that was detected. It is a B1 V star and exhibits a varying radial velocity with a period of about 5.6 d. It has a visual magnitude of 1.62 and its distance is estimated as 100 pc (ref. 2).

Since the identification of an object such as λ -Sco as a soft X-ray emitter would be of considerable importance, we decided to analyse the data obtained with the MSSL low energy (0.5–1.5 keV) X-ray telescope while the Princeton University experiment was observing the same source at ultraviolet wavelengths. The X-ray instrument on Copernicus uses several grazing incidence paraboloidal reflectors with small gas proportional counters located at their foci. A brief description of this instrument has been given elsewhere³; a more complete description is to be published shortly. The 0.5 keV to 1.5 keV telescope system, used to observe λ -Sco, used a 10 arc min. field of view for the observations reported here.

The data were obtained during some 109 h of observation and are presented in Table 1*. They are split into blocks of about 12 h. No data block showed any signal above the background. For the five blocks of data starting on September 19, 1972, the shutter intended to obscure periodically the detector windows was in operation so that the particle background could be estimated accurately. The measured background counting rates were used to produce calculated background rates for the subsequent blocks when the shutter was inoperative.

Although Bleeker *et al.* were unable to estimate the spectral shape of the radiation which they detected, the absence of any signal in their higher energy channel suggests that the spectrum is steep. We have therefore assumed a thermal spectrum, with an electron temperature of 2×10^6 K, in deriving an upper limit to the X-ray luminosity of the source. We emphasise that there is no basis in our data for this assumption but that the results of Bleeker *et al.* show no flux above 1.9 keV. The thermal continuum expression used was of the form:

$$I(E) = K \exp(-E/kT_e) \exp(-N_H \sigma(E)) \quad (1)$$

where K is a normalisation constant, T_e is the electron temperature, N_H the interstellar hydrogen column density to the source² ($\sim 7 \times 10^{19}$ atoms cm^{-2}) and $\sigma(E)$ is the weighted average photoelectric absorption cross section for the material of the interstellar medium⁴. Using equation (1) and the values of the parameters quoted above, a 3σ upper limit was obtained to the source luminosity of $4.0 (\pm 1.0) \times 10^{32}$ erg s^{-1} for the energy range 0.5 to 1.5 keV.

Bleeker *et al.* quoted a value of 2×10^{33} erg s^{-1} so it would seem that the source detected by these workers was not the star λ -Sco. It is possible, as suggested by Bleeker *et al.*, that λ -Sco is a variable. Our observations cover between 50% and 80% of the radial velocity cycle, the uncertainty being due to the lack of an exact period for these variations. If the radial velocity variations were associated with any change in the X-ray flux, it is possible, though in our opinion unlikely, that the present observations might all have coincided with 'off' states of the X-ray emission.

It is possible that λ -Sco emits soft X rays in short bursts.

* See Table 1 on page 90

If this were the case and if the outbursts were of the luminosity suggested by Bleeker *et al.* we would require about 10 min to see a signal of 3σ significance. The data were searched and no significant 10 minute blocks of data were found in 109 h of observation. In addition, a brief survey of this region by Hill *et al.*⁵, carried out about 1 h before the rocket flight of Bleeker *et al.*, failed to detect a significant signal from λ -Sco.

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Upper limit on the low energy interstellar cosmic-ray flux

DETERMINATIONS of the flux of low energy, non-solar cosmic rays extend at present down to 5 to 10 MeV for observations above the Earth¹. But the interstellar flux of cosmic rays below 1 GeV is highly uncertain because of solar modulation effects. The interstellar differential energy spectrum in the range 10 MeV to 1 GeV is usually deduced from theoretical models, by choosing more or less plausible interplanetary diffusion coefficients that will give a fit to the data. The state of sophistication of these theories is, however, still far from satisfactory, since predicted (low energy) values of the demodulated flux can vary by orders of magnitude among different authors, no experimental data on the interstellar flux having been available until now to help in selecting among different models.

In the present communication we wish to point out that there exists now an indirect experimental upper limit on this interstellar low energy flux, based on determinations of the state of ionization of the trace elements in the interstellar gas by the OAO-3 satellite Copernicus². A comparison of these results with those expected if the ionization were due to low-energy cosmic rays allows a direct upper limit to be placed on the number of ionizations per second that an interstellar hydrogen atom would suffer from cosmic rays³. This upper limit on the cosmic ray-induced ionization rate, ζ^{cr} , is

$$\zeta^{\text{cr}} < 4 \times 10^{-17} \text{ s}^{-1} \quad (1)$$

In order to obtain information on the flux from this limit, I use the definition

$$\zeta^{\text{cr}} = 4\pi \delta \int_{T_0}^{\infty} \phi(T) \sigma(T) dT \quad (2)$$

where $\phi(T)$ is the flux (particles $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹), δ is a factor allowing for secondary electrons and the effect of species with $Z > 1$, T is the cosmic-ray kinetic energy, and $\sigma(T)$ is given by the usual Bethe expression

$$\sigma(T) = 1.23 \times 10^{-20} Z^2 \beta^{-2} \cdot \{6.20 + \log [\beta^2/(1 - \beta^2)] - 0.43\beta^2\} \text{ cm}^2 \quad (3)$$

which is valid down to 0.3 MeV per nucleon.

In the case of cosmic ray protons, δ contains a factor 5/3 to allow for secondary electrons⁴, and a factor 2 to allow for species with $Z > 1$. Some of the expressions proposed for the interstellar proton flux are $\phi^{\text{p}}(T) = 1.2 \times 10^9 (T - 0.75 m_p c^2)^{-2.65}$ particles $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹ (see ref. 5) or for the proton density, $U(T) = 4\pi c \beta^{-1} \phi(T) = 9.14 \times 10^{-7} [(T + 1.5 m_p c^2)/m_p c^2]^{-2.75}$ particles m^{-3} (MeV per nucleon) (see ref. 6). If one computes with these spectra the ionization rate ζ^{cr} , using equations (2) and (3), it is found that the observational upper limit (1) is not violated, by an ample margin. This remains true even if we integrate below 0.3 MeV per nucleon, using the classical approximation cross section⁷. But the expressions quoted above for the spectrum are known to be inadequate for explaining the kinks and anomalies that appear below a few tens of MeV (refs 8, 9). To account for these, Fisk¹⁰ proposed that, after flattening in the usual manner below 1 GeV, the spectrum steepens again below ~85 MeV, in the form $\phi^{\text{p}}(T) = 4 \times 10^{11} T^{-5.5}$ particles $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹. This flux, in order not to violate expression (1), must cut off sharply at 30 MeV, or if the spectrum turns over with a slope $\propto [\sigma(T)]^{-1}$, then, the peak should be at $T > 30$ MeV. This is more stringent than the limit that would be obtained from the usual energy density arguments by a factor 2. Earlier, and also to explain the low energy kinks, Gloeckler and Jokipii¹¹ had proposed that the spectrum began rising again, below 40 MeV, as $\phi^{\text{p}}(T) = 6.2 \times 10^8 T^{-4.2}$ particles $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹. The observational upper limit here imposes a cutoff at energies $T \geq 20$ MeV. Finally, if one tried to extrapolate the high-energy flux as $\phi^{\text{p}}(T) T^{-2.65}$ down to MeV energies, and interpreted T as kinetic energy throughout (which may not be justified), the cutoff would have to come at $T \geq 50$ MeV. If one considers an arbitrary power law spectrum $\phi^{\text{p}}(T) = \phi_0 T^{-\alpha}$, taking $\sigma(T) \simeq \sigma_0 T^{-1}$ and assuming a cutoff at 1 MeV, one obtains the limit $\phi^{\text{p}}(1 \text{ MeV per nucleon}) < 5 \times 10^2$ particles $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (MeV per nucleon)⁻¹.

In the case of cosmic-ray electrons, I adopt 1/3 for the positron fraction of the total electron-positron flux, and try to interpret (1) as giving information on the electron flux. In this case $\delta = (5/3)(3/2) = 2.5$ (that is, I neglect proton, and $Z > 1$, contributions to ζ ; if this were not valid, the cutoffs derived below should be lower limits). Cummings *et al.*¹² find a good fit to the observed electron data above 10 MeV, using the diffusion-convection approximation and an assumed interstellar spectrum $\phi^{\text{e}}(T) = 3.16 \times 10^6 T^{-2.5}$ electrons $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$, which is a straight extrapolation of the flux observed above a few GeV. Condition (1) requires this flux to cutoff at $T \geq 6$ MeV, so the lower end of this assumed interstellar spectrum is dubious. In a different investigation, Cummings *et al.*¹³, from an analysis of the galactic non-thermal radio emission, derive a somewhat flatter 'nominal' spectrum $\phi^{\text{e}}(T) = 1.8 \times 10^4 T^{-1.75}$ electrons $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$ down to 80 MeV, and a possible 'high' spectrum, which below 80 MeV could be extrapolated (on the basis of their graphs) as $\phi^{\text{e}}(T) = 5 \times 10^6 T^{-1.75}$ electrons $\text{m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$. Proceeding as before, we find that these spectra cannot be extrapolated below 0.1 MeV for the 'nominal' spectrum and below 10 MeV for the 'high' spectrum. For an arbitrary power law spectrum $\phi^{\text{e}}(T) = \phi_0 T^{-\alpha}$, taking $\sigma(T) \simeq \text{con-}$

stant $= 9.3 \times 10^{-20} \text{ cm}^{-2}$ above 1 MeV (this is the transition region for the electron cross section), one obtains, assuming a cutoff at 1 MeV, an upper limit $\phi^*(1 \text{ MeV per electron}) < 1.4 \times 10^5 (\alpha - 1) \text{ electrons m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} (\text{MeV per electron})^{-1}$. The upper limit (1) thus leads to definite restrictions on the flux of low energy cosmic rays. It had been shown¹⁴ that, in order not to produce too much ^{11}B from spallation reactions, a limit could be set on the ionisation rate from protons in the range 5 to 30 MeV, $\xi_{5-30} \lesssim 6 \times 10^{-17} \text{ s}^{-1}$. This restricted the range of low energy cosmic rays that had been postulated for heating the interstellar gas to the range $T < 5 \text{ MeV}$ (see ref. 15). Since my limit (1) of $\xi < 4 \times 10^{-17} \text{ s}^{-1}$ is not restricted to an energy range, it effectively rules out cosmic rays with $T < 5 \text{ MeV}$ also. In fact, the cutoffs, when predicted, happened above this value.

I note that the limit (1) is, strictly speaking, valid within 100 pc from the Sun^{2,3}. In order to use this limit, or the cutoffs derived above, to derive conclusions about the diffuse radio, X-ray or γ -ray background, it should be kept in mind that integrations over a much longer path length are required, and a straightforward use of expression (1) may not be justified for these purposes. As far as solar modulation theories are concerned, the cutoffs derived above are probably more important in the electron case than in the proton case. This is because in the latter case the observed flux at 10 to 20 MeV is representative of a higher energy region in the interstellar spectrum⁵. But the necessity of cut-offs has been demonstrated only for the usual power law types of interstellar spectra discussed above. It may be that other types of spectra can be found to give agreement with the observed spectrum without violating condition (1). If these cutoffs are real, they may provide some information on the sources of cosmic rays. For instance, under the assumption of an isotropic, steady-state injection (such as pulsars and white dwarfs), if we assume that the low-energy particles stream with the Alfvén velocity of the ionized component of the interstellar gas, then since the lifetime of a 20 MeV portion is $\tau \simeq 1.5 \times 10^6 \text{ yr}$, the distance between sources must be $\geq 30 \text{ pc}$. Or, if low energy cosmic rays are injected by supernova events, some information on the frequency or spacing of these events may also be gained from the fact that the surviving cosmic rays must have $\tau \simeq 1.5 \times 10^6 \text{ yr}$. Alternatively, the necessity of a cutoff may not reflect some characteristic of the spatial and temporal distribution of the sources, but rather may be inherent to the acceleration mechanism itself.

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Magnetisation of comets

THE presence of magnetic fields in the plasma tails of comets is suggested by the rayed structures and helical features observed in them¹. According to the present picture of the solar wind-comet interaction (see, for example, ref. 2) it is, however, not at all clear how the interplanetary magnetic field could mix with the cometary plasma in the tail because the solar wind and the associated interplanetary field is separated from the cometary plasma by a contact surface.

Although it has been suggested that the contact surface may be liable to various instabilities such that the interplanetary magnetic field may penetrate it and establish contact with the plasma in the tail³ the viability of the process has not yet been demonstrated by any detailed analysis. It seems therefore legitimate to question if significant magnetic fields can be generated within the coma and sustained there for sufficiently long periods of time.

There are good reasons to believe that the magnetic fields of celestial bodies derive from the hydromagnetic conversion of kinetic energy into magnetic energy in their cores, although the details of the conversion mechanism are less clear. One such mechanism is provided by the well known 'magneto-hydrodynamic dynamo' (see, for example, ref. 4). An alternative and less speculative mechanism is the 'poloidal field amplification' mechanism which is founded on phenomena observed in laboratory experiments⁵ and which has been recently used for a theory of the Earth's magnetic field (H. A. and L. Lindberg, paper presented at the Geophysical and Geochemical Conference in Houston, Texas; January, 1973). Independent of which mechanism is preferred, however, the general requirements for a fluid body to be magnetised are that its volume and electrical conductivity are large enough, that there is sufficient internal kinetic energy to be converted to magnetic energy, and that there is a degree of ordering of the internal (turbulent) velocity field such as would be provided by rotation. The magnetic field H generated should be of the order

$$H = (4\pi\rho v^2)^{1/2} \quad (1)$$

where ρ is the density and v is the velocity of relative motion ('turbulent' velocity).

Clearly gas streaming out radially from a central nucleus cannot produce such magnetic field amplification, but if the nuclear region ($r \lesssim 10^4 \text{ km}$) consists of a supplementary distributed source for the coma gases as has been suggested recently by several authors^{6,7} a turbulent flow with maximum velocities of the order of thermal velocity ($\approx 1 \text{ km s}^{-1}$; ref. 7) may be expected in this region, with the necessary ordering in the turbulent velocity field provided by the rotation of the nuclear region. Taking $\langle\rho\rangle \approx 5 \times 10^{-16} \text{ g cm}^{-3}$ as a typical value in $r \lesssim 10^4 \text{ km}$ (ref. 7) and putting $v = 1 \text{ km s}^{-1}$, we obtain from equation (1) $H \approx 0.01\text{T}$, which is a significant field. Even if the turbulent velocity, v , were an order of magnitude smaller, H would still amount to about 100γ .

It is necessary to check if this magnetic field can be retained for a sufficiently long time. The time of decay τ , of the magnetic field is given by

$$\tau = 4\pi L^2 \sigma \quad (2)$$

where σ is the electrical conductivity (e.m.u.) and L is the scale length of the turbulent eddies.

In estimating the electrical conductivity of a partially ionised gas, as is the one under consideration, it is important to check if the lifetime of the electrons is sufficiently long, for otherwise the conductivity will be provided mainly by the much less mobile positive and negative ions (when it will be decreased essentially by a factor $\sim (m_i/m_e)^{1/2} \sim 100$). Although comets contain highly electronegative gases like OH the lifetime against electron attachment at the relevant densities ($n \approx 5 \times 10^7 \text{ cm}^{-3}$) is more than 10^7 s (ref. 8), about two or three orders of magnitude larger than the ionisation time scales of some of the dominant coma species (for example, CO)⁹. Consequently the appropriate conductivity is the electron conductivity, which is given by¹⁰

$$\sigma = \frac{4 \times 10^{-10} (n_e/n)}{T^{1/2} \langle Q \rangle} \text{ e.m.u.} \quad (3)$$

where $\langle Q \rangle$ is the effective collision cross section for electrons and neutral atoms and T is the electron temperature. Without a better knowledge of both the chemical composition and the photoionization processes in the inner coma it is not possible to estimate n_e/n in $r \lesssim 10^4 \text{ km}$ with any degree of certainty. If, however, we make the not unreasonable assumptions that $n_{\text{co}}/n \approx 0.1$ and that the degree of ionisation of CO in $r \lesssim 10^4 \text{ km} \approx 0.5$, then taking $T \approx 1,000 \text{ K}$ and $\langle Q \rangle \approx 10^{-15} \text{ cm}^2$, we get $\sigma \approx 5 \times 10^{-7} \text{ e.m.u.}$ Once again there is an uncertainty about the value to be ascribed to L , but if we take $L \approx 10^3 \text{ km}$ $\tau \approx 2,000 \text{ yr}$ and if we take $L \approx 10^2 \text{ km}$ $\tau \approx 20 \text{ yr}$. Even if our smallest estimate of τ is too large by two orders of magnitude we still obtain $\tau = 0.2 \text{ yr}$, which is yet of the order of the periods of coma and tail activity of a typical comet.

It therefore seems to us that a comet, when sufficiently close to the Sun, may be able to generate an appreciable internal magnetic field and that the magnetic fields observed in the tail may then result from processes analogous to those producing the Earth's magnetotail.

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Geochemical evidence for an east-dipping Appalachian subduction zone in Newfoundland

THE concept of plate tectonics explains the present behaviour of the Earth better than any rival hypothesis. Modern plate motion can be observed and measured, but unfortunately much of the evidence is ephemeral. Spreading ridges, trenches, oceanic magnetic lineaments, zones of seismic activity dipping under orogenic belts, and areas of anomalous heat flow are all destroyed or decay as the plate regime evolves. Applicability of the plate tectonic concept to the ancient past must therefore be determined by more indirect geological observations which are often open to more than one interpretation. For example, it can be inferred that melanges should develop in trenches associated with subduction¹, but not all melanges originate in this way and there is presently no easy way of typefying melanges. Thus, if the great variety of presently observed plate interactions existed in the past, geologic data may not be subtle enough to distinguish them and the record may be blurred beyond reasonable interpretation.

Given the limitations of geological data, it seems most reasonable to use the gross regional information rather than the details of local areas as a first basis for evaluating ancient plate tectonics. It also seems more reasonable to accept, at least temporarily, the simplest model suggested by these regional data rather than construct complex models to explain variations in local details.

There are several gross geochemical trends observed across presently active subduction zones. The potassium content of igneous rocks varies systematically across both island arcs²⁻⁴ and Cordilleran mountain chains⁵⁻⁷: with increasing distance from the trench, the potassium content of igneous rocks increases. There is also a systematic zonation of mineral deposits across several active orogenic zones with progressively more lithophile elements being concentrated away from the trench above the subduction zones⁸⁻¹⁰. Although these geochemical trends have not been clearly observed across all presently active subduction zones, there are no substantiated cases where a reverse zonation has been established.

Other geological features which may be systematically related to subduction zones include paired metamorphic belts¹¹, ophiolite emplacement¹², melange zones³, and piles of calc-alkaline volcanic rocks. But these features by themselves are not yet unambiguous indicators of the reality or nature of plate movements in the Paleozoic, and are especially difficult to interpret for directions of dip of paleo-subduction zones.

The various plate tectonic models that have been proposed for the Appalachian-Caledonian mountain system are summarized in Fig. 1. Most of the models presuppose plate tectonics and support the assumption with local geological data of varying reliability and significance. It is noteworthy that all the models based on empirical geochemical data are consistent, and all indicate an ancient subduction zone dipping away from the proto-North American continent in a present easterly or southeasterly direction. The models for Great Britain and Norway (ref. 15 and G. H. Gale and F. M. Vokes, unpublished) are based on the chemical composition of igneous rocks and/or the general zonation of mineral deposits. These geochemical patterns strongly resemble those across presently active subduction zones, and they represent the most direct non-paleomagnetic evidence for Palaeozoic plate movements.

Our data suggesting an easterly dipping Appalachian subduction zone in Newfoundland are derived from a study of both the geochemistry of igneous rocks and the present distribution of mineral deposits. They are supported by

other geological data.

More than 1,200 samples of granitic rocks from thirty-three plutons in Newfoundland have recently analysed in detail¹⁶. These analyses show that, irrespective of geological

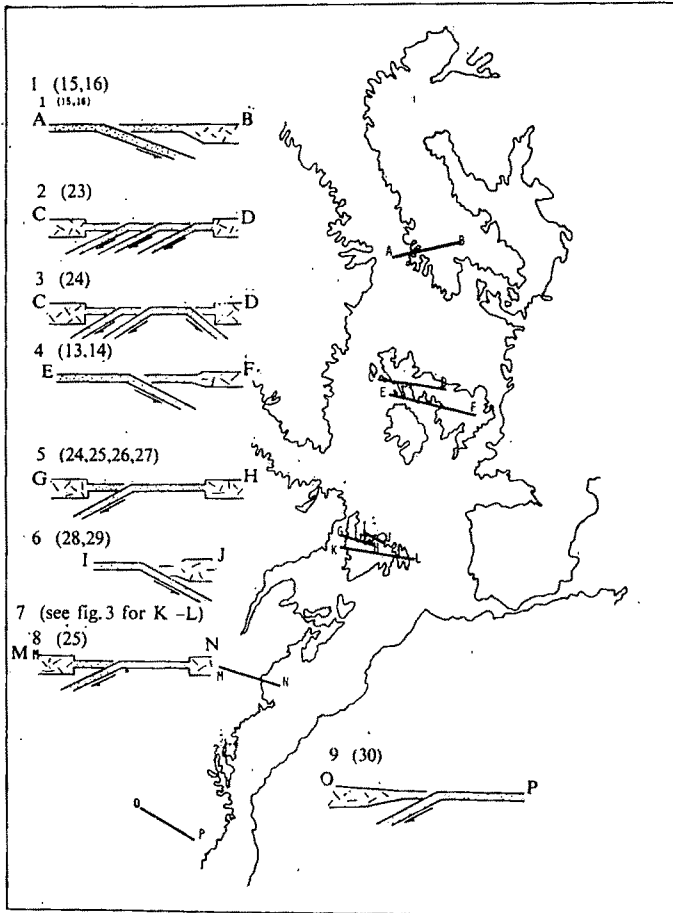


FIG. 1 Summary of plate tectonic models suggested for different sections of the Appalachians-Caledonides. Numbers in brackets next to each model refer to references. Stippled areas represent oceanic-island arc crust and lined areas represent continental crust. Map drawn from reconstruction by Bullard, *et al.*³⁰. Note that only models 1, 4 and 6 are supported by chemical data. *Unpublished results of G. H. Gale and F. M. Vokes.

age, there is a definite eastwards increase in the average potassium content of the plutons in the Central and Gander zones of eastern Newfoundland (Fig. 2). The plutons of the eastern Avalon zone do not seem to follow this trend and are therefore anomalous. This distribution of potassium is similar to that described from western North American and explained by derivation of magma from an east-dipping subduction zone⁵⁻⁷. The Avalon anomaly is comparable with the disjunct observed by Lipman, *et al.*⁷ and interpreted by them as indicating a second subduction zone. Further data may substantiate such an interpretation in Newfoundland, but our present data allow us only to make a very tentative postulation of a second subduction zone responsible for the Avalon plutonic rocks. Preliminary inspection of our data suggests that the main subduction zone dipped at a rather steep angle, perhaps as much as 60°, eastwards. A steep dip would also explain the relative narrowness of the Appalachians as compared to the Cordillera.

The zonation of Newfoundland mineral deposits¹⁷ also indicates an eastward dipping subduction zone (Fig. 3). The observed distribution is like that described by Sillitoe^{8,9} in western North and South America, Mitchell and Garson¹⁰ for the southwest Pacific and G. H. Gale and F. M. Vokes (unpublished) for Norway. Several other features of New-

foundland geology are also satisfactorily explained by a long lived east-dipping subduction zone.

The ophiolites of west Newfoundland and the Burlington Peninsula represent obducted oceanic lithosphere^{25,27}. Their westward emplacement is most simply explained by an over-

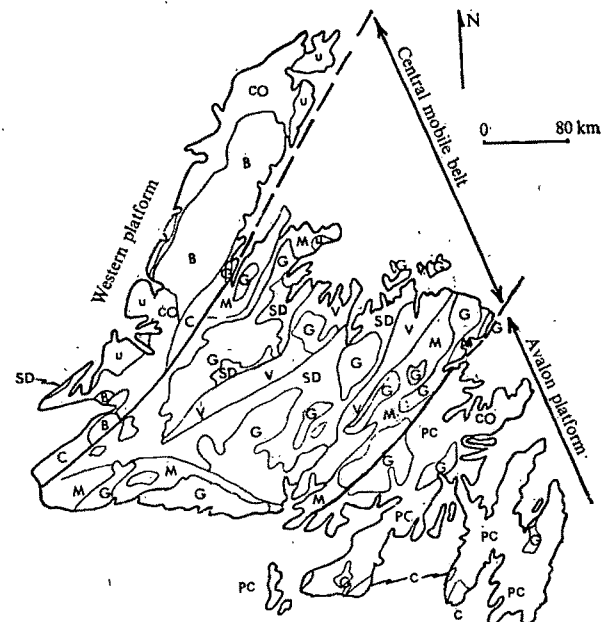


FIG. 2 Simplified geological map of the island of Newfoundland. G, Granites; u, ophiolite sequences; C, Carboniferous sediments; SD, Silurian and Devonian subaerial sandstones, conglomerates and shales; V, Ordovician pillow lavas and deep set sediments; CO, Cambrian and Ordovician sandstones, limestones and shales; M, Pre-Ordovician schists and gneisses; PC, Precambrian sediments and lavas; B, Grenville basement gneisses, granites and anorthosites.

thrusting of oceanic lithosphere onto the proto-North American continent above an east-dipping subduction zone, accompanied by an imbricate stacking up of the continental margin sediments under the ophiolites, all of which now form the west coast allochthons.

TABLE 1 Points of geological comparison between eastern and western Newfoundland

Western	Eastern
(1) Grenville Basement.	None definitely known, except possibly in the Gander Lake zone.
(2) Hadrynian plateau basalt volcanism.	Hadrynian acid ignimbrite volcanism.
(3) Fleur-de-Lys metamorphic zone.	Gander Lake metamorphic zone.
(4) Cambro-Ordovician shelf limestones.	Cambro-Ordovician Mn-Fe oxides.
(5) Ophiolites obducted onto western platform.	Ophiolites not obducted, just deformed and intrusive ultramafics.
(6) No post-Grenville granitoid rocks present.	Granitoid rocks possibly ranging from Hadrynian to Devonian in age.

The calc-alkaline piles of early and middle Ordovician volcanic rock with associated sediment in Notre Dame Bay would represent the volcanic island arcs associated with such eastward subduction.

The Gander Lake metamorphic belt would result from metamorphism along a continental margin above the subduction zone. This zone passes eastwards into the Avalon Platform continental crust, which was then a Basin and

Range type regime marked by strong faulting and bimodal basalt-ignimbrite eruptions. This Cordilleran type mountain chain might have been the long lived New Brunswick geanticline of Schuchert¹⁸. Its existence might also explain the inferred long age span of granites of the Gander Lake zone¹⁹.

The western Fleur-de-Lys metamorphic and plutonic zone would be explained according to the model of an east-dipping subduction zone as being the result of the overriding effect of oceanic lithosphere, that is subduction under oceanic lithosphere, with a resulting sinking, deformation and melting at the base of the continental crust.

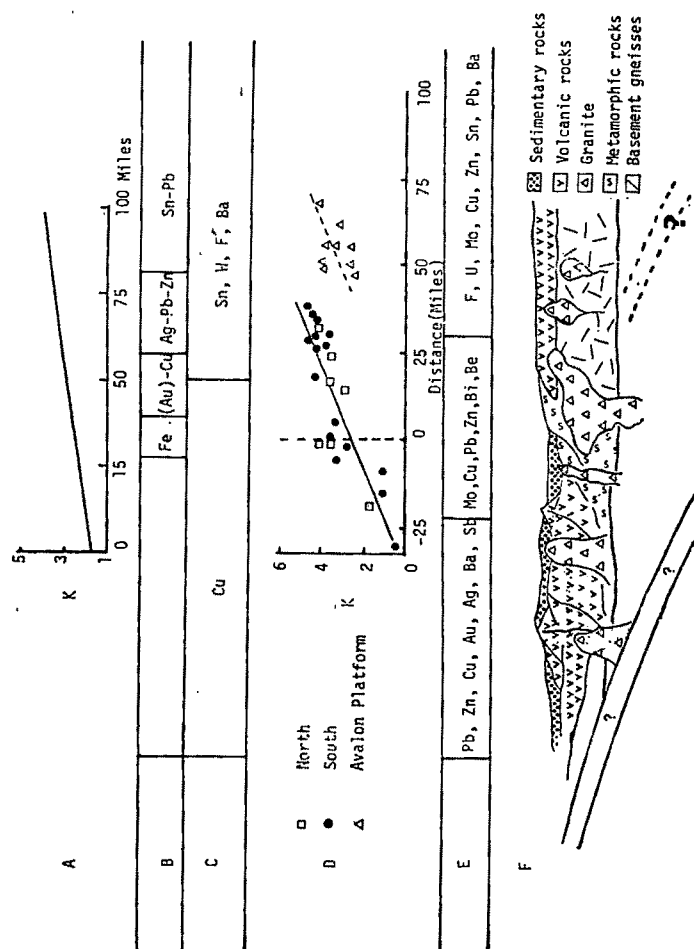


FIG. 3 Summary of geochemical and metallogenic evidence supporting an eastward-dipping Appalachian subduction zone in Newfoundland, as compared to analogous areas elsewhere. A, Variation in K across the Central Sierra Nevada Batholith (after Bateman and Dodge⁵). B, Metal zoning of western North and South America (after Sillitoe⁸). C, Metal zoning across Circum-Pacific island arcs (after Mitchell and Garson¹⁰). D, Variations in K across eastern Newfoundland. The K data points are means for individual plutons (>1,200 analyses in total), the positions of which are plotted as distance of pluton centers from the western margin of the Gander Lake metamorphic belt, which is taken as the eastern Appalachian continental margin. E, Metal zoning in eastern Newfoundland. F, Schematic plate tectonic interpretation of the data described above.

Although the gross geological symmetry of Newfoundland²⁰ can be explained by the bringing together of two

continental margins²¹ we emphasise that there is a fundamental asymmetry evident on a finer scale (Table 1, Fig. 2). It can be explained as the result of an east-dipping subduction zone. The most striking aspect of this asymmetry is that there are no post-Grenville granitoid rocks on the western platform, that is, they seem to terminate abruptly at a line approximately along the Cabot fault. This line is taken as the westward limit of subduction.

The inferred existence of a subduction zone active for more than 250 m.y. suggests that the proto-Atlantic was a rather large ocean basin.

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New approach to surface seawater palaeotemperatures using $^{18}\text{O}/^{16}\text{O}$ ratios in silica of diatom frustules

THE oxygen isotopic composition of calcium carbonate and silica deposited by organisms in water depends on the temperature and the isotopic composition of the water¹.

As deep seawater temperatures probably did not vary during the Pleistocene, the variations of the oxygen isotopic composition of the sea during this period can be followed by analysis of the $^{18}\text{O}/^{16}\text{O}$ ratio in the tests of deep benthic foraminifera². Shallow pelagic foraminifera grew in waters where significant temperature variations seem to have occurred in glacial periods. Yet the $^{18}\text{O}/^{16}\text{O}$ ratio in their carbonate tests does not show such variations. An explanation of this phenomenon lies in the ability of pelagic foraminifera to adjust the depth at which they live so as to minimise the effect of temperature variations on their biology^{2,3}.

To be able to follow oceanic surface palaeotemperatures by the variations of the $^{18}\text{O}/^{16}\text{O}$ ratio in a compound, it is necessary to be sure that the site of formation was really near the surface. Diatoms, most of which form skeletons of silica, grow in the upper 40 m because they require light for photosynthesis. Consequently, the $^{18}\text{O}/^{16}\text{O}$ ratio of their skeletons is dependent on the $\text{H}_2\text{ }^{18}\text{O}/\text{H}_2\text{ }^{16}\text{O}$ ratio and on the temperature of the surface water. Since the $\text{H}_2\text{ }^{18}\text{O}/\text{H}_2\text{ }^{16}\text{O}$ variations within a water column are very small, the $^{18}\text{O}/^{16}\text{O}$ ratio of benthic foraminifera of the same area can be used as an index of $\text{H}_2\text{ }^{18}\text{O}/\text{H}_2\text{ }^{16}\text{O}$ variations. Then, by comparing the variation of the $^{18}\text{O}/^{16}\text{O}$ ratio in silica from diatoms from different horizons of a core with that of benthic foraminiferal carbonates of the same horizons, it should be possible to calculate the surface water variations of $^{18}\text{O}/^{16}\text{O}$ ratios and temperatures during the past.

To test whether biogenous silica can be used for this purpose, it was necessary to check that it is formed in isotopic equilibrium with water, and to find the temperature relationship for $^{18}\text{O}/^{16}\text{O}$ fractionation between silica and water. For this study I analysed recent or living specimens, formed under known conditions of temperature and isotopic composition of the water.

Oxygen extraction from silica was carried out using BrF_3 by a method modified⁴ from that described by Clayton and Mayeda⁵. Detrital minerals, organic matter and the hydration water of the biogenous silica were separated from the specimens analysed. Removal of all of the detrital minerals

requires many filtrations and ultrasonic washings, and much settling and decanting⁴. Slow solution of carbonates with 0.1 M HCl is possible without oxygen isotopic exchange between the silica and the water. Organic matter is more difficult to destroy without interfering with the oxygen of the silica. But, since the organic oxygen has an isotopic composition approximately 15‰ lower, and is present as the major part of the total oxygen in recent samples, such a separation is necessary. We attribute the variations of oxygen isotopic composition seen by Mopper and Garlick⁶ on unwashed radiolaria to the slow degradation of the organic matter⁴. It is possible, using slow oxidation by NaOCl, to destroy more than 99% of the organic matter, even in fresh specimens⁷. A more serious contamination problem results from the physical nature of biogenous silica. This silica is formed in a low crystalline, highly hydrated state. In a few million years, the crystallinity increases, with progressive loss of water. This process can be accelerated by heat: The water trapped in the pores of the structure, which is in isotopic equilibrium with the environment, is volatilized by heating above 100° C in vacuum, but without apparent exchange with the oxygen of the silica. The remainder of the water (approximately 2 to 4% by weight of the silica) is partly chemically bound, in the hydroxyl form, to the silicon atoms. Heating at about 1,000° C in vacuum removes this water⁸ by increasing the crystallinity towards tridymite, cristoballite, or even quartz. To see the effect of this dehydration procedure on the isotopic composition of biogenous silica, the $^{18}\text{O}/^{16}\text{O}$ ratio of a Miocene diatomaceous earth from Cantal (France) was determined, after heating in vacuum for 1 h different samples of the same weight at different temperatures (Fig. 1). The isotopic composition is changed in a non-reproducible manner by heating between 500° C and 600° C. As most of the water is ejected in the first few minutes of heating, 1 h of treatment permits a reproducible dehydration. To ensure that the water is volatilised at constant temperature, we use a modified furnace, in which the sample reaches the plateau temperature in a few seconds. The scatter of the low temperature data indicates that rapid but incomplete isotope exchange occurs during dehydration under these conditions, partly because of interactions with residual organic matter lost at these temperatures⁹. On the other hand, the isotopic composition of specimens dehydrated at temperatures greater than 800° C is nearly constant and about 1‰ higher than that of specimens heated at temperatures below 500° C. There is only weak isotopic fractionation during exchange be-

TABLE 1 Samples and conditions of growth

Sample and origin	Water temperature °C	$\delta^{18}\text{O}$ of the water ‰ relative to snow	$\delta^{18}\text{O}$ of the samples ‰ relative to snow	$\Delta^{18}\text{O}_{\text{SiO}_2-\text{H}_2\text{O}}$ (‰) $= \delta^{18}\text{O}_{\text{SiO}_2} - \delta^{18}\text{O}_{\text{H}_2\text{O}}$	$\bar{\Delta}^{18}\text{O}_{\text{SiO}_2-\text{H}_2\text{O}}$ (‰)
Sponges					
Indian Ocean (Kerguelen)	4 ± 1	-0.25 ± 0.25	40.20 40.45	40.45 40.70	40.6
Mediterranean Sea (Banyuls)	15.5 ± 2	+1.3 ± 0.2	39.30 39.50	38.00 38.20	38.1
Atlantic Ocean					
37°48'N, 25°53'W (dredged at 700 m)	9 ± 1	+0.1 ± 0.1	38.20 38.30	38.10 38.20	38.15
36°47'N, 33°13'W (dredged at 2,000 m)	4.3 ± 0.3	+0.1 ± 0.1	40.20 40.35 40.40 40.30	40.10 40.25 40.30 40.20	40.2
Bahamas	27 ± 2	+0.8 ± 0.3	36.40	35.60	35.6
English Channel (Roscoff)	12 ± 2	0 ± 0.2	37.90 38.20	37.90 38.20	38.15
Diatoms					
Lake Pavin (France)	9 ± 3	-7.5 ± 0.1	31.40 31.60 31.90 32.0	38.90 39.10 39.40 39.50	39.2
Lake Myvatn (Iceland)	4.5 ± 2	-8 ± 1	32.15 32.45	40.15 40.45	40.3
Gulf of California 27°47'N, 111°25'W	25 ± 2	+0.6 ± 0.5	35.80	35.20	35.2

tween water and silica at these temperatures. So, if exchange occurs between silica and water during the dehydration process, the isotopic composition of the sample would remain constant. As the corresponding weight loss is only about 3%, the 1‰ ^{18}O enrichment is consistent with a water loss of isotopic composition approximately -40‰ compared to the silica. To these freshwater diatomites, $\delta^{18}\text{O} \sim 32\text{‰}$ relative to Standard Mean Ocean Water (SMOW), would correspond a water of isotopic composition -8‰ relative to SMOW, a value consistent with present freshwater composition in the sampling area. The 1‰ change in isotopic composition then can be interpreted as corresponding to a dehydration sufficiently fast to preclude isotopic exchange between water and silica. Then the isotopic composition of the silica dried above 800° C represents that of the silica as formed originally. This method has the advantage of producing crystalline, marginally hygroscopic, stable silica. The dispersion of measurements of fossil specimen isotopic composition was found to be less than $\pm 0.1\text{‰}$.

In order that silica formed under various regimens of water temperature and isotopic composition might be studied, newly deposited diatom frustules and siliceous spicules from living sponges were analysed. Preliminary results of the measurements are reported in Table 1, including the temperature and the $\delta^{18}\text{O}$ of the water during skeleton formation (as defined in ref. 9). Temperature during skeleton formation is presented as a function of the fractionation factor in Fig. 2. The regression line, based on the best defined point: $t = 5 \pm 0.3^\circ \text{C}$; $\Delta(\text{SiO}_2\text{-H}_2\text{O}) = 40.0 \pm 0.2\text{‰}$ is described by the equation: $t(^{\circ}\text{C}) = 5 - 4.1 (\delta\text{SiO}_2 - \delta\text{H}_2\text{O} - 40)$. The 1 σ uncertainty of the slope is ± 0.4 .

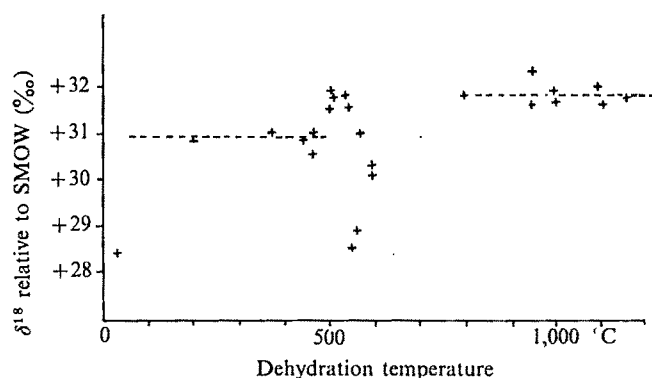


Fig. 1 Isotopic composition of the diatomite from LaBade (Cantal, France) after dehydration at different temperatures.

For palaeotemperature estimation, this uncertainty should be reduced by further analyses of warmer water specimens. But, within the present precision, the isotopic fractionation between water and either sponge spicule silica or diatom frustule silica is identical. There is then no indication of biogenic fractionation. In addition, data from freshwater samples from Lake Pavin and Lake Myvatn fell perfectly on the regression line, although these specimens were formed in water of $\delta^{18}\text{O}$ considerably different from the SMOW. The non-dependence of the isotopic enrichment of siliceous skeletons on the isotopic composition of the water in which they were formed is considered to indicate formation under conditions of isotopic equilibrium. This hypothesis is strengthened by the results of Clayton, O'Neil and Mayeda¹⁰ on the quartz water fractionation under pressure at temperatures over 200° C. We have shown⁴ that an extrapolation to room temperature of these results is valid, and gives a perfect agreement with our analysis.

To use $^{18}\text{O}/^{16}\text{O}$ in diatom silica for a Pleistocene palaeotemperature record, we must be sure this silica preserves its original isotopic composition on a scale of millions of years.

Some analyses of Miocene diatoms from fresh or marine waters exposed for a few million years to ground waters indicate no exchange in that period of time with the local ground waters⁴. But the long term variations, as observed by Degens and Epstein¹¹, restrict the application of this method to samples younger than some 10^7 yr.

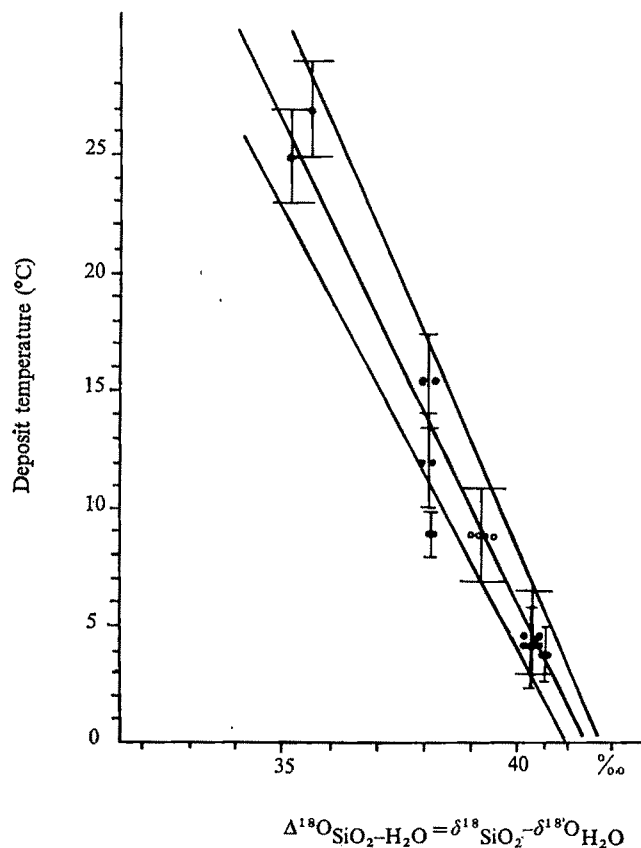


Fig. 2 Isotopic fractionation between water and silica from either diatoms or sponges, as a function of the temperature of formation. Centre line is regression line; outer lines define 1 σ envelope. \circ , Diatoms (frustules); \bullet , sponges (spicules).

In conclusion, the relationship found for the silica-water isotopic fractionation ($t = 5 - 4.1 (\delta\text{SiO}_2 - \delta\text{H}_2\text{O} - 40)$) is very similar to the known relationship for carbonates ($t = 16.9 - 4.2 (\delta'\text{CaCO}_3 - \delta'\text{H}_2\text{O})$). The slopes of these regression lines are too close to allow, through comparison of their $^{18}\text{O}/^{16}\text{O}$ ratios, the calculation of the temperature of the water in which siliceous and carbonate skeletons were formed simultaneously. But these results indicate that the measurement in sediments of $\delta^{18}\text{O}$ in frustules of diatoms and in associated tests of benthic foraminifera may be used to follow the variations of surface water temperature during the Pleistocene.

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Glacial advance relative to volcanic activity since 1500 AD

THERE may be a connection between the periodicity of glacial advance and the occurrence of volcanic eruptions. Lamb¹ has presented a graph which shows a similar curve for Arctic sea ice off Iceland and great Icelandic volcanic eruptions since 870 AD and Bray² found the number of maximum advances of alpine glaciers since 1680 AD is significantly related ($\chi^2 = 7.8$, $P < 0.01$) to intervals with a volcanic dust veil index of 1,000 or greater. The volcanic dust estimates have now been extended to 1500 AD (ref. 1) and in this paper I analyse the relationship between these estimates and records of maximum glacial advance from 1500 AD to the present.

Glacial activity was assessed by a compilation of maximum advance dates, defined as all advances which reached the terminal moraine or its near vicinity. Only dates based on historical observations or on distortions in the growth rings of trees standing at the time of glacial advances were selected, in order to ensure a maximum accuracy. A total of 128 dates were compiled from summary studies of which 16 dates were from North American trees²⁻⁵ and 112 were from European historical observations^{2,6-8}. The North American and European dates were synchronous.

The compiled maximum glacial advance dates are shown in Table 1 at 20 yr intervals together with Lamb's estimate of the amount of volcanic dust in the atmosphere of the Northern Hemisphere (from Table 7a of ref. 1). Both the glacial advance and volcanic dust data have a series of similar peaks during the 17th, 18th and 19th centuries, with lower values in the 16th and 20th centuries. There is a significant positive correlation ($r = +0.43$, $P < 0.05$) between the glacial and volcanic activity data in Table 1 which supports the results of the previous study². The basic data summarised in Table 1 show a tendency for glacial advance to follow major volcanic eruptions which may reflect the lag between the time of ice buildup and its maximum advance (as noted below).

A mechanism for the apparent relationship between glacial advance and volcanic eruption has been suggested²: After a major volcanic eruption, there is a global temperature decline from atmospheric dust for up to 3 or 4 yr or more². During this decline, there are colder springs and summers¹ and as a result, some precipitation which usually falls as warm storms over the icefields (thus promoting ablation), falls instead as snow. These snowfalls can increase the summer albedo of the icefield up to four times (albedo old ice ~ 20%, new snow ~ 80%) and together with a decrease in the number of warm storms is a major influence² in producing a strong positive ice balance and a consequent glacial advance.

This proposed sequence of major volcanic eruptions followed by several years of cold summers and then by glacial advance is supported by historic and phenologic data. Of

TABLE 1 Maximum glacial advances and atmospheric volcanic dust in the Northern Hemisphere 1500 to 1960 AD

Year AD	No. of maximum glacial advances	Dust Veil Index (DVI)
1500-19	0	650
1520-39	0	150
1540-59	1	1,250
1560-79	1	0
1580-99	3	1,090
1600-19	9	1,810
1620-39	5	825
1640-59	8	1,475
1660-79	3	1,675
1680-99	6	1,480
1700-19	12	1,220
1720-39	1	815
1740-59	9	1,280
1760-79	6	550
1780-99	4	1,940
1800-19	16	2,545
1820-39	12	3,180
1840-59	24	1,140
1860-79	2	1,120
1880-99	5	1,690
1900-19	1	770
1920-39	0	0
1940-59	0	400

the 102 late wine harvests which occurred on or after October 10 from 1500 AD to 1880 AD in France (data converted to a national basis from Appendix 12 of ref. 6), 25 occurred in the 59 yr with volcanic eruptions with DVI (Dust Veil Index) of 300 or greater ($\chi^2 = 6.3$, $P < .01$). A significant relationship ($\chi^2 = 27.0$, $P < 0.001$) with volcanic eruption was also determined for the 128 maximum glacial advance dates of which 111 occurred within 24 yr after the 23 volcanic eruptions with a DVI of 1,000 or greater from 1500 to 1970 (Table 2). The distribution of the lag between volcanic eruption and glacial advance shown in Table 2 is in agreement with studies³ of the interval between ice buildup in the icefield and subsequent maximum glacial advance.

Sequences of volcanic eruptions followed by cold summers and then glacial advance are shown in Table 3 which summarises: (1) all the intervals of 10 yr or less with volcanic eruptions with a total DVI of at least 1,000 (from Appendix I, ref. 1); (2) all years of cold summers in England (ref. 1, page 494) and France (ref. 6, pages 56, 58, 60) and years of famine and food shortage due to cold summers in western Europe and Japan (ref. 2, Table VII; ref. 1, page 494; ref. 6, pages 66-68, 92); and (3) the 128 glacial advance dates. The most notable sequences in Table 3 are those following the two largest volcanic eruptions, 1815 and 1835. The 1815 eruption was the peak of a series of eruptions from 1811 to 1817. Cold springs and summers, late wine harvests and food shortages occurred from 1812 to 1817 and included the "year without a summer" in 1816. A major glacial expansion began in 1814, maximum advances were reached from 1817 to 1824 and ice retreat began in 1819.

TABLE 2 Maximum glacial advances in the Northern Hemisphere in relation to global volcanic eruptions $\geq 1,000$ DVI, 1500-1970 AD

No. of years following eruption $\geq 1,000$ DVI	No. of maximum glacial advances		χ^2
	Expected	Observed	
0-4	29.3	37	2.0
5-9	25.9	41	8.8
10-14	17.7	23	1.7
15-24	20.7	10	5.5
25-44	19.8	8	7.0
45-75	14.3	9	2.0
			27.0

TABLE 3 Sequences of volcanic eruption, cold summers and maximum glacial advance, 1500–1970 AD

Volcanic eruptions, Year AD	global DVI	Cold summers, late harvests, food shortages, Europe and or Japan Year AD	Maximum glacial advances, Northern Hemisphere Year AD	No.
(1912	500)	1912		
1895*, 1902	2,300	1903	1898–1910	2
1883*, 85, 86, 88	3,150		1883–94	3
1875*	1,000	1879	1881	1
(1868–70	1,000)†	1866–69		
1856, 61	1,500		1860–71	2
1845*, 46	1,800	1840s	1840–55¶	24
1835	4,000	1833–39		
1821, 22	1,100			
1811–1815*, 17	4,800	1812–17	1817–24¶	26
1795*, 96, 98, 99	2,200		1807–09	2
1779, 83*, 86	4,050	1782–87	1786–89	3
1763, 66*, 68	4,450	1760s	1771–80	5
1752*, 54, 55	2,500	1755	1756–60	3
(1730–1736	700)	1740–42	1736–43	9
1717, 21, 24	1,600	1725		
1707, 12	1,600	1709, 1711–17	1712–20¶	10
1693, 94	1,800†	1692–98	1694–1700	6
1680*	1,000			
1673*	1,000	1672–75	1676–80¶	5
1660*, 64	2,900			
(1646, 50	800)	1646–50	1653	1
1636, 38, 40, 41*, 46	2,900	1639–43	1640–44¶	7
1625, 31	1,400	1627–33	1628–30¶	5
1614*	1,000	1618–21		
1601*, 06	1,550		1610	2
1593*, 97	1,300	1594–97	1589–1601¶	10
1586*	1,000	1586–87		
1553*–54*	1,000	1555	1576	1
		1527–29	1546	1
1500*	1,000			

Years which had eruptions > 1,000 DVI; †DVI could be as high as 3,000 to 3,500; ‡DVI estimated from temperature anomalies; ¶underlined glacial phases are those emphasized by Ladurie for western Europe (pp 195, 211 ref. 5).

The 1835 volcanic eruption, together with subsequent eruptions in 1845 and 1846, was followed by cold springs and summers, late wine harvests and food shortages in 1833–39 and the 1840s with a major glacial advance period occurring from 1840 to 1855. All the glacial expansion phases with five or more advances were preceded by major volcanic eruptions except that of 1736–43 which was preceded by a period of cold summers, food shortages and severe famine in 1740–42, but by only a minor volcanic eruption of 700 DVI from 1730–36. The 1527–29 food shortage period was apparently not related to a preceding volcanic eruption.

The role of global temperature (perhaps controlled, in part, by solar activity²) in the proposed volcanic eruption-glacial advance sequence may be to determine the number and magnitude of glacial advances. This may be shown by the relatively lower number of advances following volcanic eruptions during the generally warmer period¹¹ of the mid to late 18th century compared with the preceding and following centuries which had colder temperatures¹¹ and more numerous glacial advances following volcanic eruption. It may also explain the lower number of ice advances during the warmer temperatures of the 12th and 13th centuries² when there was a volcanic activity peak¹, compared with the large number of ice advances following volcanic eruption during the colder temperatures² of the 17th and 19th centuries.

There is evidence that, since the Pleistocene, periods of intense volcanic activity may have coincided with major ice advance phases. There were three well dated major volcanic activity periods¹ in the Southern Andes: from 50–450 BP, from 2150 to 2450 BP and from 4720 to 5450 BP. The dates for the three main Neoglacial ice advance phases have been

listed⁹ as 50–450 BP, 2600–2800 BP and 4500–5000 BP. Two of these Neoglacial phases almost exactly correspond with the Andean volcanic activity periods and the second Neoglacial phase, which peaked from 2600–2800 BP extended from 2300 to 2900 BP (refs 9, 12–17). A fourth Andean volcanic period has been tentatively dated around 8950 BP and there was an ice advance phase^{17–19} around 9200 to 9300 BP.

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Quantitative Spectrochemical Analyses of Feldspars by Ion Bombardment

WHEN the surface of a solid target is bombarded with energetic ions or neutral atoms, spontaneous emission of ultraviolet and visible radiation is observed. When the radiation is analysed by a spectrometer, it is shown to contain characteristic lines of the constituent elements in the solid^{1–4}. It has also been shown that this radiation is caused by the decay of excited atoms ejected from the target surface^{4,5}. The photon emission process is more efficient in insulating targets than metallic targets. This is because of the existence of radiationless de-excitation processes which compete with radioactive decay in the case of metals but not in the case of insulators^{1,6}. The intensities of the spectral lines are directly proportional to the bombarding ion beam current⁷, that is, directly proportional to the number of sputtered particles since the sputtering yield (sputtered atoms per incident ion) for a particular material is invariant if the ion energy is constant providing that the surface conditions do not alter during sputtering. In a previous report¹, one of us suggested that the photon emission phenomenon may provide a new method for chemical analysis of solids, and subsequently White *et al.*³ demonstrated the detection sensitivity, for example 1 part per 10⁶ for sodium in a silicate glass, which could be achieved with this technique. This communication describes some quantitative analyses of a number of feldspars. The analyses were made by comparing the intensities of the emission lines of the major constituents in these materials. In our analyses, we assume that diffusion does not occur to an appreciable extent so that the relative sputtering rates are directly proportional to the atomic concentrations in the target.

Table 1 Feldspar Analyses. Number of Ions on the Basis of Thirty-two Oxygens

	112 (standard) Microprobe	IBSCA	190 Microprobe	Chemical	Chemical	191 IBSCA	191 Microprobe	IBSCA	297 Microprobe
Si	10.086	9.34	9.423	9.341	9.293	11.23	11.132	9.92	9.990
Al	5.916	6.63	6.523	6.628	6.659	4.75	4.863	6.05	5.995
Fe	0.029	0.037	0.056	0.033	0.059	0.015	0.0	0.026	0.027
Ca	2.005	2.63	2.605	2.628	2.616	1.01	0.903	2.09	2.064
Na	1.720	1.32	1.324	1.248	1.192	2.90	2.939	1.83	1.772
K	0.105	0.050	0.026	0.053	0.028	0.091	0.133	0.074	0.088
An	52.4	65.8	65.8	67.2	68.9	25.2	22.7	52.3	52.6
Ab	44.9	33.0	33.5	31.5	30.3	72.5	74.0	45.8	45.2
Or	2.7	1.2	0.7	1.3	0.7	2.3	3.3	1.9	2.2

	287 IBSCA	Microprobe	109 IBSCA	Microprobe	184 IBSCA	Microprobe	111 IBSCA	Microprobe
Si	9.14	9.004	11.79	11.717	11.62	11.805	8.50	8.436
Al	6.81	6.984	4.20	4.297	4.37	4.229	7.46	7.520
Fe	0.038	0.063	0.006	0.0	0.005	0.013	0.038	0.057
Ca	3.04	2.967	0.34	0.298	0.233	0.192	3.52	3.666
Na	0.88	0.937	3.61	3.626	3.14	2.694	0.439	0.250
K	0.076	0.035	0.041	0.011	0.626	0.951	0.041	0.0
An	76.0	75.3	8.5	7.6	5.8	5.0	88.0	93.6
Ab	22.0	23.8	90.3	92.1	78.5	70.2	11.0	6.4
Or	1.9	0.9	1.0	0.3	15.7	24.8	1.0	0.0

Provided that self-absorption is negligible (because the 'vapour' density of absorbing atoms is low), then we have the condition for spectrochemical analyses (intensity is directly proportional to concentration).

The apparatus, which we shall refer to as IBSCA (ion beam spectrochemical analyser⁸), is identical to the one described previously¹ with the exception that in place of the medium quartz spectrograph, the light was analysed in the present work by a grating monochromator with a cooled photomultiplier as detector. The light intensity, being very low, was measured by a standard a.c. chopping technique.

The analysis procedure was carried out as follows. First, the results of the electron microprobe analysis of a particular feldspar 112 was chosen as standard for all the subsequent analyses. Then the ratios of the intensities of Si 2882 : Al 3093, Si 2882 : Al 3944, Si 2882 : Al 3962, Si 2882 : Fe 3737, Ca 3934 : Na 5890, Ca 3968 : Na 5890, Ca 4227 : Na 5890, and K 7665 : Na 5890, were taken. By comparing the intensity ratios of each unknown feldspar with those of 112, we could calculate the concentration ratios. Using the fact that in a unit cell of feldspar there are thirty-two oxygen atoms, sixteen atoms shared by Si, Al and Fe, and four atoms shared by Ca, Na and K, we could work out each individual atomic concentration in terms of thirty-two oxygens. Unfortunately, we could not analyse the oxygen content in the feldspars accurately because of the presence of oxygen impurity both in the argon gas used for producing the ion beam and in the target chamber, possibly due to contamination of water vapour; otherwise the oxygen 7772 line could have been used as a reference line. (This contamination can probably be removed by using a 4A zeolite and more efficient trapping with liquid nitrogen.) We have therefore chosen to carry out our analyses in the manner described rather than comparing intensities of a particular line directly across samples since the present method minimises uncertainties which may arise due to a change in sputtering conditions, for example variations in ion current and chamber pressure. Since the specimen holder in the present apparatus only allows one sample to be analysed at a time, the changing of samples might therefore cause sputtering conditions to vary from one sample to another. The method of analysis adopted, however, assumes only that the sputtering conditions did not vary during an experimental run on a particular sample.

The analyses of seven feldspars are given in Table 1. The electron microprobe analyses by N. Ware (private com-

munication) are also shown for comparison. Electron microscopy has shown that the substructure of these feldspars is on a scale considerably less than 1 μm ^{9,10}. The electron microprobe has a spot size of about 10–100 μm diameter whereas, in IBSCA, the area under investigation was determined by the monochromator slit width which was 150 μm . It is therefore expected that the microprobe and IBSCA should produce similar results. As can be seen from Table 1, the two sets of results are in good agreement. Slight variations in compositions are expected, because different samples (of the same materials) were used for the IBSCA and microprobe analyses. Two chemical analyses¹¹ of feldspar 190 are also shown in Table 1 to demonstrate the degree of uncertainty involved in the determination of the composition of feldspars.

During our investigation, the following conclusions on IBSCA as an analytical instrument have emerged. (1) The apparatus costs considerably less than other comparable analytical instruments because of its simplicity. The estimated cost of constructing such an apparatus is approximately from \$4,000 to \$10,000 depending on the degree of sophistication required. (2) Multi-element analysis is easy to carry out and no elaborate preparation of the sample is required. (3) In the present method, the area of investigation is effectively selected by the variable slit of the monochromator. If the slit can be replaced by a small circular aperture, it will become a microprobe with mechanical scanning facilities. Its ultimate performance as a microprobe is limited by a spatial resolution of ~ 10 μm assuming the photon-emitting sputtered particles leave the target surface with energies ≤ 10 eV¹. (4) The apparatus can detect light elements down to hydrogen. (5) As the method essentially carries out analysis of the surface, it can be used to measure the concentration profile in a solid as successive surface layers are sputtered away. (6) The line spectra contain very few lines compared to ordinary spectrochemical analysis by arc discharge but more than just resonance lines. This makes identification of elements a very rapid and easy process. (7) With suitable refinements, IBSCA can be used as a research instrument due to its probe characteristics. But as it stands, it can be a very useful field or industrial instrument due to its great simplicity.

In view of the possibility of significant applications, a new IBSCA apparatus using a duoplasmatron ion source to deliver a stable and high current density ion beam and a multiple-target specimen chamber to facilitate rapid and direct com-

parison of intensities is under construction for improved performance.

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BIOLOGICAL SCIENCE

Inhibitor of vesicular stomatitis virus transcriptase in purified virions

VESICULAR stomatitis virus (VSV) particles contain an RNA-dependent RNA polymerase which gives rise *in vitro* to RNA molecules of the same size and polarity as the mRNA found *in vivo*¹⁻³. It is not clear which structural protein(s) represents the enzymatic activity. Only five proteins (L, G, N, MS, and M, with possibly some additional minor constituents) are detected in the virions⁴, and several studies have suggested that neither the G protein (glycoprotein surface spike) nor the M protein (membrane protein), nor even associated membrane lipids are necessary for transcriptase activity⁵⁻⁷.

Active polymerase has been reconstituted from two inactive fractions, one containing the ribonucleoprotein core and the other, the remainder of the detergent-solubilized proteins⁸. In such a case some components of the virion, in addition to the polymerase itself, may modify or control the transcriptase activity. We report here that VSV particles contain a specific inhibitor of the virion polymerase.

The presence of such an inhibitor was suggested by the results presented in Fig. 1. Polymerase activity measured as a function of virus concentration, was strongly inhibited when virus concentrations were high. This was not due to an early shut-off of *in vitro* activity since under these conditions the kinetics of incorporation were similar for both high and low concentrations, slowing down between 3 and 5 h after the start of the reaction. Various modifications of the reaction cocktail established that none of the components were limiting in inhibiting conditions. Both VSV serotypes—Indiana and New Jersey displayed similar concentration-dependent inhibition, which was the same for virus grown in HeLa as well as BHK₂₁ cells. Further purification of the virus on tartrate-density gradients caused some loss of inhibitory activity for New Jersey VSV but not

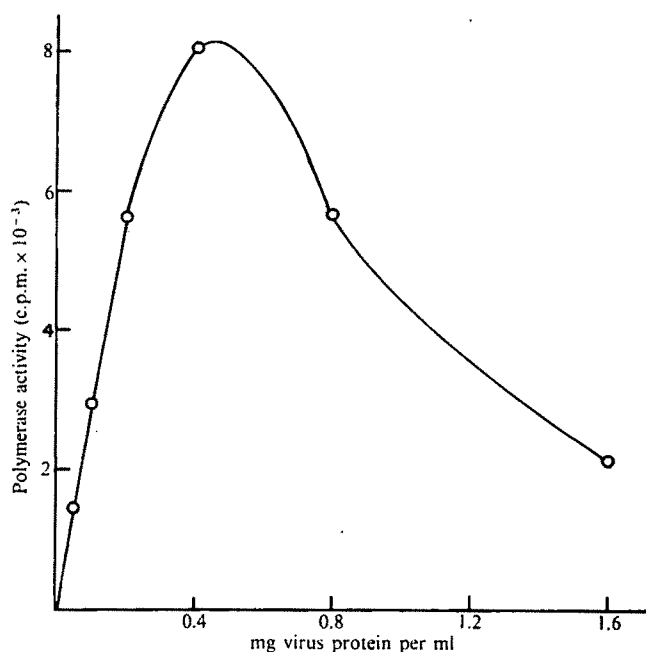


Fig. 1 Virion polymerase activity as a function of virus concentration. The cells, virus sources, and growth of the virus were as described in detail elsewhere⁸. Monolayers of BHK₂₁ cells were infected at a multiplicity of infection of 10 with the Indiana VSV serotype. The media were collected after 12-16 h at 37°C and clarified by spinning at 5,000g for 20 min. All operations were carried out at 4°C. The supernatants were spun at 19,000 r.p.m. for 3 h in a Spinco type 19 rotor. The virus pellets were resuspended in small volumes of 0.05 M Tris-Cl, pH 7.5, buffer and sonicated briefly to disrupt clumps. The sonicates were layered on 12 ml 15-45% sucrose gradients in TK buffer (10 mM Tris-Cl, pH 7.7, 100 mM KCl) and spun at 32,000 r.p.m. for 35 min in the Spinco SW 41 rotor. The single visible band of standard particles (no defective interfering particle bands under these conditions) was collected with a Pasteur pipette and stored on ice until assayed. The concentration of virus protein was determined using the relationship 1 mg virus protein per ml equals 3 A₂₆₀ units (M. E. Reichmann, personal communication). Transcriptase assays were carried out by mixing equal volumes of virus suspension diluted in 25% sucrose in TK buffer with equal volumes of polymerase cocktail. The final concentration of the components in the total reaction mixture was as follows: 50 mM Tris-Cl, pH 7.7; 100 mM KCl; 5 mM MgCl₂; 0.1% mercaptoethanol; 0.02% Nonidet P-40; 1 mM each of ATP, GTP, and CTP; 0.1 mM cold UTP; 2.5 μCi-ml⁻¹ ³H-UTP; and approximately 10-15% sucrose. ³H-UTP incorporation was measured on 50 μl samples after 2 h of incubation at 28°C by the method of Blatti *et al.*⁹ Under these conditions incorporation of 1 nmol of UMP is equivalent to 14,000 c.p.m.

for Indiana (see below). The phenomenon could also be demonstrated by adding ultraviolet-irradiated virus (inactive in the polymerase reaction) to standard active virus (Table 1) or by adding defective interfering particles which have little or no transcriptase activity¹⁰ (data not shown). Thus, transcription by the inhibiting virus preparation was not a prerequisite for inhibition.

Preliminary experiments with virus purified through sucrose gradients only, suggested that the inhibitor was serotype-specific. Table 1 shows the results of one such experiment in which Indiana and New Jersey ultraviolet-irradiated virus preferentially inhibited their own serotype polymerase. Several experiments, however, showed either partial specificity or none. Some of this variability may have been caused by contaminating nucleases, which are often present in virus preparations purified on sucrose gradients but not in those density-banded on tartrate gradients (L. P. Villareal, personal communication).

When the virus was purified further through tartrate density gradients the results shown in Fig. 2 were obtained

TABLE 1 Homotypic compared with heterotypic inhibition of *in vitro* transcriptase with sucrose-purified VSV

	c.p.m.
Indiana control	5975
Indiana control + ultraviolet-Indiana	1396
Indiana control + ultraviolet-New Jersey	5390
New Jersey control	5442
New Jersey control + ultraviolet-Indiana	5778
New Jersey control + ultraviolet-New Jersey	2433

Indiana and New Jersey VSV were purified as described in Fig. 1. Virus suspensions in TK-buffered sucrose were irradiated in small nitrocellulose cups at a distance of approximately 6 cm from an ultraviolet sterilizing lamp (G8T5-General Electric) for 6 min. Irradiated virus never incorporated more than 1-2% of controls. Transcriptase assays were carried out as in Fig. 1 except for a final ^3H -UTP concentration of $25 \mu\text{Ci ml}^{-1}$. Control viruses were adjusted to $45 \mu\text{g}$ virus protein per ml and ultraviolet-viruses, to $675 \mu\text{g}$ virus protein per ml final concentration. The c.p.m. listed refer to total incorporation per $30 \mu\text{l}$ sample after 4.5 h at 28°C .

repeatedly. Clearly under these conditions, Indiana VSV polymerase was inhibited specifically by homotypic ultraviolet-irradiated virus but not by heterotypic virus, which was somewhat stimulatory (Fig. 2A). New Jersey VSV polymerase, however, showed a different pattern of response when either serotype was added. Both New Jersey and Indiana ultraviolet-irradiated virus caused early inhibition of incorporation followed later by stimulation (Fig. 2B). Thus, tartrate-purified New Jersey virus did not cause sustained inhibition of either serotype polymerase. It is not known why homotypic inhibition is lost for the New Jersey serotype virus on further purification.

The extent of the inhibition and stimulation of New Jersey polymerase in Fig. 2B, as well as the heterotypic stimulation of Indiana polymerase (Fig. 2A), varied in different experiments, but the homotypic Indiana inhibition was always considerable and readily observed. It is not clear whether these various phenomena are reflections of one or more activities in the preparations.

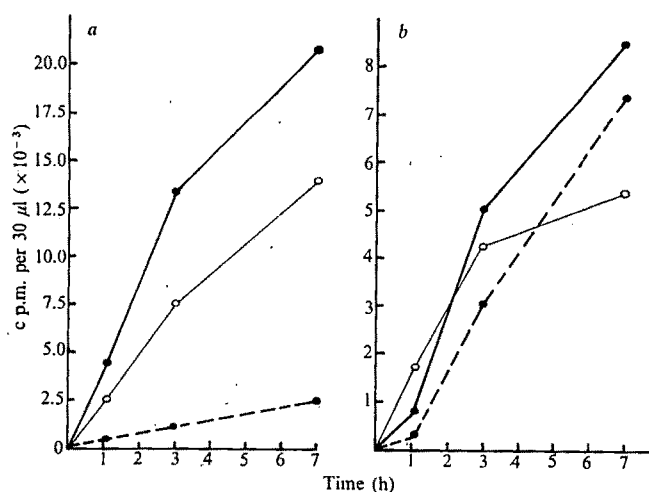


FIG. 2 Serotype specificity of transcriptase inhibition with tartrate-purified virus. Indiana and New Jersey VSV were further purified by layering on 15-45% NaK-tartrate gradients and spun in the Spinco SW 41 rotor at 37,000 r.p.m. for 3 h at 4°C . The virus bands were recovered and dialysed against TK buffer. Transcriptase assays were carried out as in Table 1. Control preparations contained $50 \mu\text{g}$ virus protein per ml and the ultraviolet preparations were added to a final concentration of $800 \mu\text{g ml}^{-1}$. A, Indiana polymerase; B, New Jersey polymerase; \circ , control; \bullet , plus ultraviolet-irradiated New Jersey virus; $-\bullet-$, plus ultraviolet-irradiated Indiana virus.

The effect of graded amounts of ultraviolet-irradiated virus was examined in experiments carried out in parallel to those of Fig. 2. The totals after 7 h of incorporation were used to construct the curves shown in Fig. 3. In agreement with the previous results, Indiana showed strong homotypic inhibition, while New Jersey polymerase was not inhibited by either serotype. The inhibition reached a maximum of approximately 80% at a particle ratio of approximately 5 ultraviolet-irradiated virus:1 active virus. On the other hand, stimulation of polymerase activity did not reach a maximum with increasing amounts of irradiated virus.

We conclude that the inhibitory activity has some type specificity. It is therefore likely that this phenomenon is

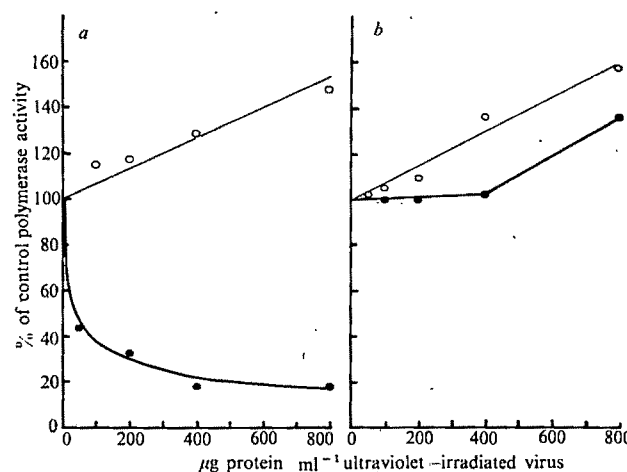


FIG. 3 Polymerase activity versus ultraviolet-virus concentration. The activities plotted are explained in the text and in the legend for Fig. 2. \circ , plus ultraviolet-irradiated New Jersey virus; \bullet , plus ultraviolet-irradiated Indiana virus.

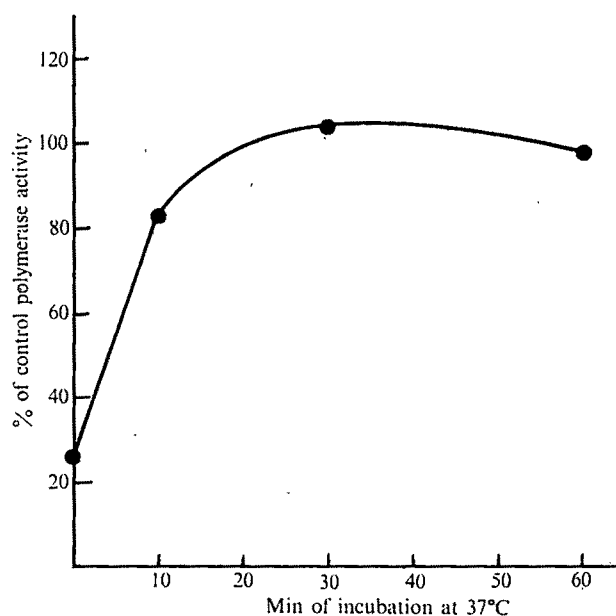


FIG. 4 Loss of inhibitor activity at 37°C in the presence of Nonidet P-40 detergent. Indiana VSV purified through sucrose and tartrate gradients was ultraviolet-irradiated as before and incubated at 37°C in TK buffer with 0.02% Nonidet P-40 at a concentration of $800 \mu\text{g}$ virus protein per ml. This suspension was then assayed for inhibitor activity as described in Fig. 2 at a final concentration of $400 \mu\text{g}$ per ml. The totals after 6.25 h of incorporation were used to construct the curve shown in the figure.

TABLE 2 Effects of predigesting virions with trypsin on polymerase and inhibitor activities of Indiana VSV

	c.p.m.
Control virus 60 $\mu\text{g ml}^{-1}$	4,309
+ 65 $\mu\text{g ml}^{-1}$ ultraviolet-virus	3,387
+ 130 $\mu\text{g ml}^{-1}$ ultraviolet-virus	2,630
+ 260 $\mu\text{g ml}^{-1}$ ultraviolet-virus	1,463
+ 65 $\mu\text{g ml}^{-1}$ trypsinized ultraviolet-virus	3,009
+ 130 $\mu\text{g ml}^{-1}$ trypsinized ultraviolet-virus	2,303
+ 260 $\mu\text{g ml}^{-1}$ trypsinized ultraviolet-virus	1,629
Trypsinized virus 60 $\mu\text{g ml}^{-1}$	3,367

A 0.2 ml suspension of sucrose-purified Indiana VSV containing 2.5 mg virus protein per ml and 100 $\mu\text{g ml}^{-1}$ trypsin was incubated at 37°C for 2 h. The suspension was then passed through a 10 ml column of Bio-Gel A-5m, 100–200 mesh (Bio Rad), equilibrated with TK buffer. Control virus was handled in the same way except that trypsin was added after the 2 h incubation at 37°C. The transcriptase assays were carried out as in Table 1. The c.p.m. listed refer to incorporation per 30 μl samples after 3 h at 28°C. The kinetics of incorporation were similar to those shown in Fig. 2A for both control and trypsinized virus.

controlled, directly or indirectly, by some genetically determined virus component.

Initial investigations of the heat stability of the Indiana inhibitor gave the following results. Purified virions suspended in TK-buffered sucrose solution showed no loss of inhibitory activity when incubated for 2 h at 37°C. A temperature of 100°C for 1 min under the same conditions destroyed the inhibitor. If the virus was first disrupted with 0.02% Nonidet-P40 and incubated at 37°C as above, the inhibitor activity was lost with a half-life of approximately 5 min (Fig. 4), suggesting that the inhibitor irreversibly modifies a component of the polymerase under the conditions of the *in vitro* assay.

To determine whether the inhibitory activity is present on the surface of the virion, possibly as the spike protein,

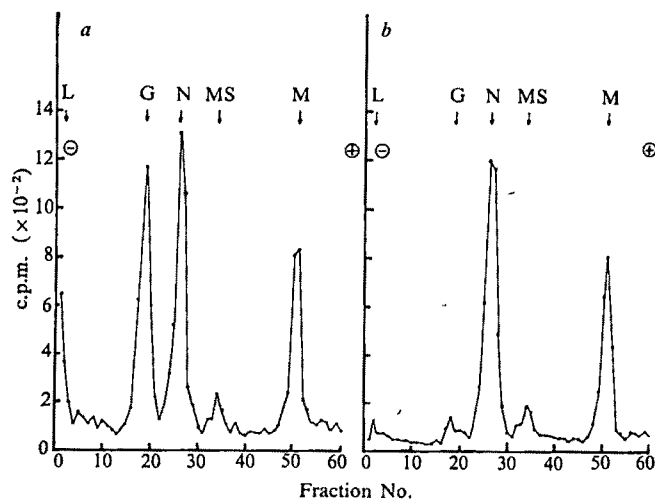


FIG. 5 SDS gel electrophoresis of control and trypsinized Indiana VSV. Identical samples to those used in Table 2 were mixed with a small amount of high specific activity ^3H -amino acid (phenylalanine, tyrosine, and valine)-labelled Indiana VSV (supplied by Dr John Mudd). After processing as in Table 2, the samples were mixed with SDS and mercaptoethanol to 1% final concentration. They were then boiled for 30–60 s and dialysed against 10 mM sodium phosphate buffer, pH 6.8, 0.1% mercaptoethanol, 0.1% SDS, and boiled again. 0.3 ml samples were then subjected to electrophoresis in 7.5% acrylamide, 0.37% bis-acrylamide gels, in 0.1 M sodium phosphate, pH 6.8, 0.1% SDS buffer, for approximately 15 h at 90 V. The gels were crushed in a linear fractionator and the fractions counted in a Triton X-100 scintillation cocktail. A, control; B, trypsin-treated.

the Indiana virus preparation was digested with trypsin to remove the surface glycoprotein without affecting other virus components¹¹. The resultant skeleton was separated from trypsin by passing through a small column of Bio-Gel A-5m. This structure was then tested for remaining polymerase and inhibitory activity. Table 2 shows that approximately 80% of the polymerase activity and essentially all the inhibitory activity was retained in the skeleton structure. Parallel controls, in which a small amount of high specific activity ^3H -amino acid-labelled virus was added before trypsin digestion, were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Fig. 5 indicates that more than 90% of the glycoprotein was removed. This experiment provides further evidence that the inhibitor is a virus-specific component, for trypsin treatment probably removes surface contaminants. Furthermore, the intact glycoprotein is ruled out as a candidate for the cause of the inhibitory activity.

We conclude from these data that purified VSV contains a specific inhibitor of the virus transcriptase. Two lines of evidence support this conclusion. First, the inhibition shows some degree of serotype specificity, and second, the inhibitor is present within the core structure of the virion produced by trypsin digestion. It is clear that in many studies where VSV transcriptase was measured *in vitro*, this inhibitory activity must have been present.

We can only speculate about the role of this activity *in vivo*. If the inhibitor is a VSV protein, as is most likely, the synthesis of this protein in the infected cell could lead to a switch from transcriptase to replicase activity. The common polymerase 'core' hypothesis for these two distinct activities is attractive as no new major proteins have been detected in infected cells apart from structural proteins^{12,13}. Another role for this activity could be to shut off host cell RNA synthesis.

It is interesting that Astell *et al.*¹⁴ reported that a reovirus structural protein, σ_3 , inhibits reovirus transcriptase when added back to subviral preparations lacking this protein. The relationship, if any, between these different inhibitors is not clear.

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Structural homology of the glutamine amidotransferase subunits of the anthranilate synthetases of *Escherichia coli*, *Salmonella typhimurium* and *Serratia marcescens*

ANTHRANILATE synthetase (AS), the enzyme catalysing the initial reaction unique to tryptophan biosynthesis, chorismate + glutamine \rightarrow anthranilate, has been characterised from a number of bacterial genera¹. In *E. coli*²⁻⁴, *Salmonella typhimurium*⁵⁻¹⁰, and *Aerobacter aerogenes*¹¹, this enzyme and anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase (PRT) exist in a complex that also performs the second reaction of tryptophan biosynthesis, the conversion of anthranilate + 5-phosphoribosyl-1-pyrophosphate \rightarrow phosphoribosyl anthranilate. The AS-PRT complex of these bacterial genera is composed of two non-identical polypeptide components, each with a molecular weight of approximately 60-65,000. Component I of the complex can convert chorismate + $\text{NH}_3 \rightarrow$ anthranilate but cannot use glutamine as amino donor. Component II of the complex is bifunctional; it provides the glutamine amidotransferase (GAT) and the PRT activities. In *Serratia marcescens*¹²⁻¹⁴, *Bacillus subtilis*¹⁵⁻¹⁶, *Acinetobacter calcoaceticus*¹⁷⁻¹⁸, and various species of *Pseudomonas*¹⁹⁻²⁰, AS is not associated with PRT, which is a distinct protein. The AS of these bacteria consists of two non-identical subunits of different sizes. The large subunit, approximately 60-70,000 in molecular weight, functions as does component I of *E. coli*, *S. typhimurium* and *A. aerogenes* while the small subunit contributes the GAT activity. The molecular weights of the GAT subunit of *S. marcescens*^{12,14}, *Pseudomonas putida*^{19,20}, *B. subtilis*¹⁵, and *A. calcoaceticus*¹⁸ have been estimated to be 21,000, 18,000, 16,000 and 14,000 respectively. The PRT of *S. marcescens* has a molecular weight of 45,000, which is smaller than that of component II of *E. coli* and *S. typhimurium* by an amount equivalent to the molecular weight of a GAT subunit¹⁴.

The two functions of the component II of *E. coli* and *S. typhimurium* have been shown to be associated with different segments of the polypeptide chain. Proteolytic digestion of either the component I-component II complex

of *E. coli* and *S. typhimurium*⁹ or purified component II of *S. typhimurium*²¹ destroys PRT activity, but produces a component II fragment with GAT activity which is similar in size to the GAT subunit of *S. marcescens*. Genetic and mutational studies have further indicated that the amino terminal third of the component II of *E. coli*²² and *S. typhimurium*²¹ is responsible for the GAT activity while the remaining two thirds of the polypeptide provides PRT activity^{21,23}.

It has been suggested^{12,14,21} that the bifunctional component II of *E. coli*, *S. typhimurium* and *A. aerogenes* arose by the fusion of two genes, one coding for a GAT and the second coding for PRT. To examine this possibility, we have isolated and compared the amino terminal sequences of the GAT subunit of *S. marcescens* and the component II of *E. coli* and *S. typhimurium*.

For ease in purification and subsequent subunit separation, we isolated the GAT portion of component II of *E. coli* and *S. typhimurium* from partial proteolytic digests of intact component I-component II complexes. As indicated below, the amino terminal sequence of the GAT portion of the component II of *E. coli* is identical with that of intact component II of this organism. Presumably this is also true of *S. typhimurium*. The AS of *S. marcescens* was isolated intact and the subunits then separated. The details of the purification of the component II fragments and the *S. marcescens* complex and subunits will be described elsewhere. On the basis of SDS gel electrophoresis analyses we estimate that the molecular weights of the component II fragments of *E. coli* and *S. typhimurium* are 23,000 while that of the GAT subunit of *S. marcescens* is 21,400. The molecular weight of the component II fragment of *S. typhimurium*^{9,21} had previously been estimated as 24,000 and that of the GAT subunit of *S. marcescens*¹² as 21,000.

Automatic Edman degradation of the purified polypeptides was performed with a Beckman sequencer²⁴. Phenylthiohydantoin-amino acids were identified by gas-liquid chromatography²⁵ and (or) amino acid analysis²⁶ after hydrolysis of phenylthiohydantoin-derivatives with HCl or HI. The amino acid sequences of the component II fragments of *E. coli* and *S. typhimurium* and the intact GAT subunit of *S. marcescens* deduced from several degradation runs are shown in Fig. 1.

	5	10	15	20
<i>E. coli</i>	Ala-Asp-Ile-Leu-Leu-Leu-Asp-Asn-Ile-Asp-Ser-Phe-Thr-Tyr-Asn-Leu-Ala-Asp-Gln-Leu-			
<i>S. typhimurium</i>	- - - - - - - - - - - - - - - Trp - - - - -			
<i>S. marcescens</i>	- - - - - - - - - Val - - - - - - - - - Val - - - - -			
	25	30	35	40
	Arg-Ser-Asn-Gly-His-Asn-Val-Val-Ile-Tyr-Arg-Asn-His-Ile-Pro-Ala-Gln-Thr-Leu-Ile -			
	- Thr -			
	- Ala Ser - - - Gln - - - - - - - - - Gln - Gly - - - Val Ile -			
	45	50	55	60
	Glu-Arg-Leu-Ala-Thr-Met-Ser-Asn-Pro-Val-Leu-Met-Leu-Ser-Pro-Gly-Pro-Gly-Val-Pro-Ala-			
	Asp - - - - - ? - - - - - X X X X X X X X X			
	- - - Gln Gln - ? Gln - - - - - - - - - - - Ala - Val			

Fig. 1 Comparison of the amino-terminal sequences of the component II fragments of *E. coli* and *S. typhimurium* and AS-GAT subunit of *S. marcescens*. Residues of the *S. typhimurium* or *S. marcescens* proteins which differ from those in *E. coli* are given, while identity to the *E. coli* residue is indicated by a dash (-). Automatic Edman degradation of the component II fragment of *S. typhimurium* was programmed for 52 cycles only, while 61 residues were sequenced with the proteins of *E. coli* and *S. marcescens*. The amino acid residue at position 47 in the *S. typhimurium* and *S. marcescens* sequences could not be identified.

(Details will be published elsewhere.) To establish that the sequences of the component II fragments are identical to the amino terminal sequences of intact component II, we performed fifteen steps of automatic Edman degradation on isolated, intact, component I-component II complex of *E. coli*. The sequence attributable to component II was identical to that of the component II fragment.

It is evident from the alignment in Fig. 1 that the three sequences are homologous. The sequences of *E. coli* and *S. typhimurium* differ from that of *S. marcescens* by 23% (14/60 residues) and 27% (14/51 residues), respectively. It can also be seen that the sequences are in the same register throughout the 51 residue region compared, and that all three begin with the identical sequence of 8 residues. In view of these similarities it seems likely that the genes for the three GAT regions have homologous nucleotide sequences in the regions coding for the amino terminal segment of the respective polypeptides. Thus, it is highly probably that the GAT region of the bifunctional component II polypeptide of *E. coli* and *S. typhimurium* had the same evolutionary origin as the GAT subunit of the AS of *S. marcescens*. Functional homology of the GAT subunits of *E. coli* and *S. marcescens* is demonstrated by the observation that the GAT subunit of the latter species will complement *E. coli* component I to form an active hybrid molecule that can use glutamine as an amino donor¹⁴. In some organisms, for example *B. subtilis* and *A. calcoaceticus*, in which AS contains a small GAT subunit and is not associated with PRT, the GAT subunit appears to participate in a second biosynthetic reaction, that of *p*-aminobenzoate formation^{19,18}. It is not known if this is also true of the GAT subunit of the AS of *S. marcescens*. In view of all these observations, we can hypothesize, in agreement with earlier suggestions^{12,14,21}, that the evolutionary path resulting in the development of the bifunctional component II of *E. coli* and *S. typhimurium* was as follows. In an ancestral organism tryptophan biosynthesis preceded with NH₂ rather than glutamine as amino donor in anthranilate formation. Subsequently a GAT subunit, perhaps involved in *p*-aminobenzoate synthesis, acquired the ability to complex with a polypeptide analogous to AS component I, thereby permitting the use of glutamine, a more suitable amino donor. Then a copy of the gene for this GAT subunit was translocated into a tryptophan operon, adjacent to the gene coding for PRT. Coincident with the integration event, or later, the GAT and PRT genes became fused.

Two additional observations may be cited which lend support to some of the stages in the above scheme. First, in *E. coli*²⁷ it seems that there is a distinct gene for the GAT subunit of the *p*-aminobenzoate synthetase complex. Second, the GAT region of the gene coding for component II of *E. coli* is not essential for anthranilate formation^{23,28,29}. This suggests that addition of glutamine-utilizing activity to AS simply increased the efficiency of conversion of chorismate to anthranilate rather than providing the ability to perform a new, essential biosynthetic reaction.

It is not yet known whether the structural gene for the GAT subunit of the AS of *S. marcescens* is in the same operon as the structural genes for either the large subunit of AS, or for PRT¹⁴. In *B. subtilis* the structural gene for the small GAT subunit is unlinked to a single operon containing the genes for all the other proteins involved in tryptophan biosynthesis¹⁹. In *A. calcoaceticus* the structural gene for the GAT subunit is in a gene cluster also containing the genes for PRT and indole glycerolphosphate synthetase¹⁷. This gene cluster is not linked to the structural genes for any of the other proteins of tryptophan biosynthesis¹⁷. In *E. coli* and *S. typhimurium* all the genes are in one operon and the gene for component II follows immediately after the gene for component I, with the genetic region specifying the GAT portion of component

II, the amino terminal third of this polypeptide, closest to the gene for Component I^{5,21,22}.

It should be noted that the fusion of two structural genes with the retention of both enzyme activities has been demonstrated in studies with the histidine operon of *S. typhimurium*³⁰. Both fused and separate forms of functional polypeptides occur for other reactions of tryptophan biosynthesis e.g., in *E. coli*³¹⁻³³, *S. typhimurium*³³, *A. aerogenes*³³ and *S. marcescens*^{34,35}, phosphoribosylanthranilate isomerase and indoleglycerol phosphate synthetase activities are associated with a single polypeptide chain while in *A. calcoaceticus*¹⁷, *B. subtilis*³⁶ and *Pseudomonas putida* these activities reside in separate polypeptide chains.

A final point seems pertinent. The postulated series of evolutionary stages—from no GAT, to one GAT participating in two pathways, to two GATs, each specific for a pathway, can be considered analogous to the evolution in higher organisms of multiple genes coding for functionally and structurally similar proteins.

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DNA Repair Monitored by an Enzymatic Assay in Multinucleate Xeroderma Pigmentosum Cells after Fusion

XERODERMA pigmentosum is a rare cutaneous disease of man displaying an autosomal recessive pattern of inheritance¹. The most conspicuous clinical symptom exhibited by homozygously afflicted individuals is hypersensitivity of the skin to solar radiation. Typically, the disorder eventuates in a high incidence of multiple carcinomas and frequently becomes terminal after the onset of metastatic epithelioma². Two clinical syndromes characterise the disease: (i) the more common classical form manifested solely by the cutaneous abnormalities and (ii) the De Sanctis-Cacchione (DSC) syndrome in which severe neurological complications, including mental retardation, accompany the skin malignancies².

Ultraviolet radiation ($\lambda < 320$ nm) induces cyclobutyl dimers between adjacent pyrimidines in the DNA of human cells. Cumulative evidence³ indicates that these otherwise deleterious photoproducts are removed from cellular DNA by an excision-repair process. The repair mechanism probably proceeds by the following sequence of enzymatic steps: (i) intrastrand incision proximal to the photoproduct; (ii) excision of the lesion perhaps within an oligonucleotide; (iii) repair replication to fill in the resulting single-strand gap, and (iv) strand rejoining³. Excluding several unexplained cases^{4,5}, primary fibroblasts derived from many genetically unrelated patients with either form of xeroderma pigmentosum are deficient in the excision repair of ultraviolet-induced damage^{3,6-9}. By analogy to the well documented excision-repair properties of ultraviolet-sensitive bacteria, the primary molecular defect in xeroderma pigmentosum most likely impairs the functioning of an ultraviolet-specific endonuclease¹⁰⁻¹³—the human analogue of the repair enzyme mediating the initial incision reaction in the bacterium *Micrococcus luteus*¹⁴.

In spite of an apparent obstruction at the same stage in repair, the genetic defects in the two clinical forms of xeroderma pigmentosum are complementary. Using somatic cell hybridisation, de Weerd-Kastelein *et al.*¹⁵ demonstrated that in binuclear cells containing one nucleus from each clinical form, DNA repair returns to that found in normal, repair-proficient cells. Thus, the ultraviolet-induced response is normal only when the binuclear cells possess a heterozygous combination of nuclei. To gain further insight into the fidelity of complementation, particularly at the biochemical level, we have used an *in vitro* enzymatic assay¹² to follow the disappearance of dimer-containing sites from the DNA of ultraviolet-damaged, multinucleate heterokaryons of xeroderma pigmentosum strains. The rationale behind the assay is the selective ability of an ultraviolet-specific endonuclease purified from *M. luteus* to produce single-strand scissions at sites containing pyrimidine dimers (referred to as nuclease-susceptible sites) in DNA extracted from ultraviolet-damaged cells.

As strongly suggested by inferential evidence¹², the assay monitors the presumed initial incision event. Use of this assay in combination with one measuring a late step in repair (that is, repair replication) may therefore permit us to detect in xeroderma pigmentosum hybrids any peculiarities in the excision-repair process stemming from the occurrence of genetic complementation.

The experiments reported here were performed with three human strains: AH⁹, established from a healthy volunteer; XP4RO derived from a classical xeroderma pigmentosum patient and XP25RO¹², procured from a De Sanctis-Cacchione donor. Monolayer cultures of primary fibroblasts were cultured at 37° C in Roux flasks containing thymidine-free F12 medium¹⁶ supplemented with 15% (v/v) foetal calf serum (Gibco). When required, radionuclides were incorporated into endogenous DNA by incubating cultures for 36–48 h in growth medium containing either 0.5 $\mu\text{Ci ml}^{-1}$ ³H-methyl thymidine (specific activity 2 Ci mmol⁻¹) or 1.0 $\mu\text{Ci ml}^{-1}$ 2-¹⁴C-thymidine (specific activity 53 mCi mmol⁻¹). (Both radiochemicals were purchased from the Radiochemical Centre, Amersham, Great Britain.)

Multinucleate cells were formed according to the procedure of Harris and Watkins¹⁷. Trypsinised cells of the two chosen strains were mixed in a 1:1 ratio at a final concentration of 2×10^6 cells per ml and incubated with ultraviolet-inactivated Sendai virus (final titre 500 haemagglutinating U ml⁻¹). This mixture was kept for 4 min at 4° C and after shaking at room temperature incubated for 20 min at 37° C. The fused fibroblasts were then seeded in Falcon Petri dishes (diameter 9 cm, each dish receiving the equivalent of 2×10^6 single cells) and incubated overnight at 37° C.

All subsequent procedures including those pertaining to (i) the administration of far ultraviolet light (chiefly 254 nm radiation; incident exposure rate 9 ergs mm⁻² s⁻¹); (ii) the enzymatic assay to monitor the disappearance *in vivo* of dimer-containing sites from cellular DNA, and (iii) ultraviolet-induced repair replication as measured by isopycnic centrifugation in sodium iodide gradients, were performed as described in detail earlier^{12,18}.

Incubation of ultraviolet-irradiated cultures for selected periods before performing the *in vitro* enzymatic assay enables us to trace the time course for the disappearance of nuclease-susceptible sites from the DNA of XP4RO/XP25RO hybrids and thus determine whether genetic complementation occurs at the presumed initial step in excision repair. The results of such an experiment are presented in Fig. 1. Fused cultures containing XP4RO/XP25RO heterokaryons can indeed rid their DNA of nuclease-susceptible sites; for example, after exposure to 125 ergs mm⁻² of germicidal light ~35% of the sites initially produced have been removed in 15 h while a smaller percentage—up to 20%—is eliminated during the same interval following an incident fluence of 250 ergs mm⁻². In sharp contrast, non-complementary preparations of XP4RO/XP4RO and XP25RO/XP25RO, like the corresponding unfused cell cultures¹², are at most only marginally proficient at attacking these sites containing pyrimidine dimers. It would appear, however, that the capacity for eliminating the sites from ultraviolet-damaged DNA has only partially returned after cell fusion of XP4RO was XP25RO; that is by the fifteenth hour after irradiation with 125 erg mm⁻² the xeroderma pigmentosum hybrid population has removed ~50% of those eliminated by normal cells. This intermediary level of site removal observed in the XP4RO/XP25RO hybrids is in excellent agreement with the percentage of the total nuclei in the same fused cultures present in multinucleate cells (that is ~55%). And since the enzymatic assay monitors not just the complementary heterokaryons but rather the entire cell population (also comprising mononucleate unfused cells and non-complementary fused ones containing two or more nuclei derived from the same strain), it seems reasonable to conclude that within the sub-population of XP4RO/XP25RO heterokaryons the ability to execute site removal has been restored to that proficiency displayed by normal cells.

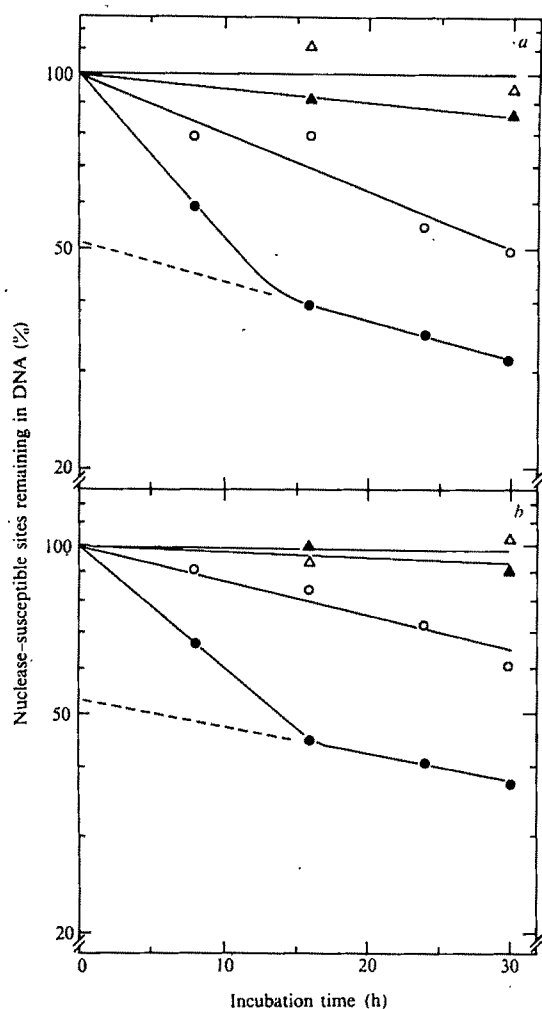


Fig. 1 Relative rate of disappearance of nuclease-susceptible sites from the DNA of various fused human cultures after exposure to measured fluences (*a*, 125 ergs mm⁻²; *b*, 250 ergs mm⁻²) of ultraviolet radiation. Tritium-labelled cells of two selected strains were artificially fused as were two parallel ¹⁴C-labelled preparations. After incubation overnight 254 nm light was administered to the ³H-labelled hybrid cultures; immediately thereafter both the ultraviolet-damaged and the undamaged cultures were incubated in pairs for requisite times. The two types of radioactive cells were collected, pooled, and mixed thoroughly before cell lysis and *in toto* extraction of the irradiated and un-irradiated DNA. Two aliquots of the purified DNA were next incubated under conditions described before¹², one sample in the absence and the other in the presence of the ultraviolet-specific endonuclease from *M. luteus*. The number of nuclease-susceptible sites in the ultraviolet-injured DNA was determined retrospectively by sedimenting the two incubated DNA samples through alkaline sucrose gradients (40,000 r.p.m. at 20° C for 150 min in a SW 50.1 rotor). Finally the ensuing DNA profiles were analysed by a digital PDP8/1 computer to quantify the incidence of single-strand scissions directly attributable to endonucleolytic attack by the *Micrococcus* enzyme. (See ref. 12 for details concerning this computation.) The data for each curve were normalised by expressing the number of sites detected in the incubated samples as a percentage of the value (taken as 100%) found in the corresponding un-incubated sample. Each experimental point is the arithmetic mean of at least two independent determinations. Combinations of fused strains: ●, AH/AH; ○, XP4RO/XP25RO; ▲, XP4RO/XP4RO; △, XP25RO/XP25RO.

Self-hybridisation of a single strain (that is XP4RO to XP4RO) does not noticeably alter the repair properties of human fibroblasts. In unfused cell cultures the repair-proficient AH strain displays two-component kinetics for site removal at fluences of 65 and 125 but not at 250 erg mm⁻² (ref. 12), but in fused cell populations of AH these kinetics are also readily

demonstrable at 250 erg mm⁻² (Fig. 2). Although these discrepancies are of little relevancy to the interpretation of our data, they suggest a difference in the general morphological geometry of the fibroblasts after heterokaryosis. In comparison with the mononucleate cells, the fused cells, particularly those of high multinucleicity, attach but do not spread out on the Petri dish to the same degree before ultraviolet irradiation with the result that the effective biological fluence (measured retrospectively by the enzymatic assay) is consistently less than the calibrated incident fluence. This dosimetrical discrepancy presumably reflects partial shielding of nuclei by various cellular constituents absorbing 254 nm radiation.

In the same experiment we also studied complementation between strains representing the classical xeroderma pigmentosum and the DSC syndromes by assessing in the fused cell populations the magnitude of repair replication. This indicator of repair activity is interpreted to reflect the cumulative occurrence of the third step in excision repair in which single-strand 'repair patches' are inserted at infrequent intervals along the damaged DNA molecule⁶.

Fused preparations of normal AH cells served to simulate hybrids in which the extent of complementation was maximal while hybrid cultures of XP4RO/XP4RO and XP25RO/XP25RO were assayed to establish the residual level of repair exhibited by each XP strain in the absence of complementation. Figure 2 clearly demonstrates that XP4RO and XP25RO cells are considerably more proficient at repair replication when they are combined in multinucleate heterokaryons than when self-fused in homokaryons. As suggested above to account for the partial restoration of site

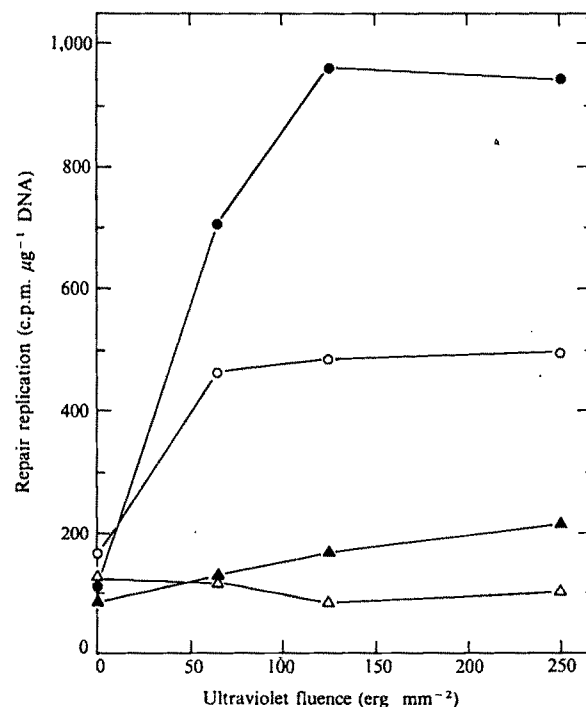


Fig. 2 Relative levels of DNA repair replication in artificially fused human fibroblasts as a function of incident ultraviolet fluence. After cell fusion and incubation overnight, the unlabelled cultures were grown in BrUdR (2 μg ml⁻¹) and FdUdR (10⁻⁶ M) for 2 h, exposed to indicated fluences of 254 nm radiation, and immediately labelled with ³H-methyl TdR (10 μCi ml⁻¹), BrUdR (2 μg ml⁻¹), FdUdR (10⁻⁶ M) and hydroxyurea (10⁻³ M) for an additional 3 h. The DNA was extracted *in toto* from each sample and centrifuged to equilibrium in neutral NaI gradients (38,000 r.p.m. at 20° C for 44 h in a Type 40 fixed-angle rotor). The isopycnic gradients were generated and analysed to measure the amount of repair replication (expressed as specific activity, that is, c.p.m. μg⁻¹ DNA, in DNA of normal density). Combinations of fused strains: ●, AH/AH; ○, XP4RO/XP25RO; ▲, XP4RO/XP4RO; △, XP25RO/XP25RO.

removal in the same preparations, the fractional amount of repair replication detected in the XP4RO/XP25RO hybrid cultures compared with that found in normal cells is an effect ascribed to the fusion condition in which only a fraction of the nuclei are present in multinucleate heterokaryons. Hence, in accord with similar studies reported elsewhere^{15,19}, after artificial union classical xeroderma pigmentosum and DSC strains mutually compensate for their respective genetic deficiencies.

Our results strongly suggest that as a result of genetic complementation multinucleate hybrid fibroblasts of XP4RO and XP25RO, human strains derived from classical xeroderma pigmentosum and DSC donors, respond to insult from ultraviolet radiation by executing *bona fide* excision repair with complete fidelity. These heterokaryons seem to exhibit normal kinetics for both the disappearance of nuclease-susceptible sites and repair replication, parameters probably measuring initial and late steps in excision repair. Furthermore, there is no rampant accumulation of single-strand scissions in the DNA of the damaged hybrid cultures during post-radiation incubation, as the incidence of strand breaks in ultraviolet-damaged DNA from incubated as compared with non-incubated samples was negligible. The most reasonable interpretation of our data is that the two clinical forms of xeroderma pigmentosum reflect mutations in different genetic complementation groups coding for the initiation of excision repair.

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Measurement of Defects in Ultraviolet-irradiated DNA by the Kinetic Formaldehyde Method

ULTRAVIOLET irradiation of DNA converts a portion of the adjacent pyrimidine bases into cyclobutane dimers of the form $\hat{C}\hat{C}$, $\hat{C}\hat{T}$ and $\hat{T}\hat{T}$ ¹. As a consequence, hydrogen bonding and base stacking at these sites are disrupted, and localised denatured regions may appear. (Ultraviolet-induced denaturation has been reviewed in ref. 2.) Treatment of DNA with formaldehyde results in a preferential reaction at these denatured sites (or defects) because formaldehyde reacts only with free but not hydrogen-bonded amino groups³.

The theory developed by Trifonov *et al.*⁴ and Lazurkin *et al.*⁵ called the kinetic formaldehyde method (KF method), relates the rate of the reaction of formaldehyde with DNA to the concentration of defects in the DNA. Since formaldehyde unwinds the DNA as it reacts, the rate of reaction can be followed by determining the change in helicity from the change in the absorbance A_t at some time t , where

$$\theta = (A_{t_{final}} - A_t) / (A_{t_{final}} - A_{t_{initial}})$$

in which $A_{initial}$ and $A_{t_{final}}$ are the initial and final absorbances respectively. The relationship between θ and t is given by

$$(-\ln \theta) / t = 2cv + pv$$

where c is the concentration of initial defects per base pair, v is the velocity of unwinding at a defect site, and p is the rate constant for the initiation of new defect sites. A plot of $(-\ln \theta) / t$ against t gives a straight line with slope pv and intercept of $2cv$ on extrapolation to $t=0$. The initial rate of unwinding is defined as the intercept, $I=2cv$.

Defects can be single or double strand chain breaks or pyrimidine dimers. Experimental evidence shows that v is the same for both single and double strand breaks⁶. Little direct evidence, however, is available to support the basic assumption made in those studies that v for pyrimidine dimers is the same as for chain breaks, and the assumptions made in calibrating the KF method for pyrimidine dimers have been criticised².

Shafranovskaya *et al.*⁶ showed that for ultraviolet (254 nm) fluences $>10 \text{ J m}^{-2}$ the rate of formaldehyde reaction with irradiated DNA was six times slower than expected on the basis of numbers of dimers present in the DNA. The result was interpreted in terms of clustering of the dimers such that six dimers, for example, would be expressed as a single defect site. The origin of such clustering was proposed to be long range energy transfer (over as many as several thousand base pairs) resulting in preferential de-excitation at or near the site of an initially formed pyrimidine dimer, which serves to block further migration of the energy. An alternative possibility is that a break and a dimer are not equivalent sites, because of the constraints in unwinding an unbroken helix, and hence react with formaldehyde at very different rates.

We have designed experiments to test the assumption used by Shafranovskaya and coworkers that dimer and chain break defect sites react with formaldehyde at the same rate. To do so we used ultraviolet-endonuclease to convert pyrimidine dimer sites into chain breaks. This enzyme recognises pyrimidine dimers and makes nicks at the dimer sites⁷⁻⁹. A comparison of the rates of formaldehyde reaction before and after enzyme treatment should demonstrate any differences between the velocity of unwinding, v , associated with dimers and with chain breaks.

Tritium-labelled DNA was isolated from *Escherichia coli* B3 by the method of Marmur¹⁰ and dissolved in 0.01 M phosphate buffer, pH 7. Irradiation was done with the output (mostly 254 nm) from a 15-W low-pressure mercury lamp, in some cases in the presence of 0.03% H_2O_2 to produce chain breaks. Photolysis of H_2O_2 occurs with a quantum yield of unity, creating hydroxyl radicals which in turn make single strand

Table 1 Kinetic Formaldehyde Analysis of DNA containing Chain Breaks and Dimers

Ultraviolet fluence (J m ⁻²)	H ₂ O ₂	M _w (daltons × 10 ⁻⁶)	Breaks per base pair (× 10 ³)	UT/T (× 10 ²)	TT/T (× 10 ²)	PyPy per base pair (× 10 ³)	I (min ⁻¹ × 10 ³)	V = I/2C (min ⁻¹)
0	+	7.2	0	—	—	—	0	0
100	+	2.5	0.33	—	—	—	1.75	2.7 ± 0.5
500	+	0.63	1.93	—	—	—	6.35	1.7 ± 0.5
1,000	—	—	—	0.6	2.4	7.7	2.5	0.16
2,500	—	—	—	0.6	4.4	12.8	7	0.27

DNA was irradiated with 254 nm ultraviolet light in the presence, or absence of 0.03% hydrogen peroxide. Molecular weights were determined by sedimentation in an alkaline sucrose gradient. Single strand chain breaks were estimated assuming $M_n = 0.5 M_w$ (M_n , number-average; M_w , weight-average molecular weight) and $(M_n^0/M_n) - 1 = \text{No. breaks per molecule}$ (M_n^0 , initial molecular weight). Defects per base pair were calculated assuming 1,500 base pairs per 10⁶ daltons. PyPy per base pair does not include CC. The intercepts (I) were determined by extrapolation of $(-\ln \theta/t)$ back to $t=0$; I for the unirradiated sample was $1.5 \times 10^{-3} \text{ min}^{-1}$, which value was subtracted from all the intercepts to give the values shown. In addition, the intercepts for the peroxide treated samples were corrected for the small contributions estimated to come from dimers produced during irradiation.

chain breaks in the DNA¹¹. Photoproduct yields and single strand chain breaks were determined as described previously¹². The rate of reaction with formaldehyde (2.53%) was measured at 255 nm and at 54° C with a Beckman DU equipped with a Gilford model 2000 spectrophotometer attachment. The initial absorbance (A_{initial}) of the solutions was measured immediately after adding formaldehyde. After 90 min at 54° C, the temperature was raised to 90° C for 10 min and the final absorbance (A_{final}) was measured. The absorbance data were treated as described by Trifonov *et al.*⁴ and Lazurkin *et al.*⁵ to obtain I , the initial rate of unwinding.

To convert pyrimidine dimers into chain breaks, the ultraviolet-endonuclease isolated from *Micrococcus luteus* (a gift from W. L. Carrier) was allowed to react with the irradiated DNA for 30 min at 37° C. The enzyme was removed from the DNA before the formaldehyde reaction by extraction with an equal amount of 24:1 chloroform:alcohol. The DNA was then dialysed, with three changes, for 24 h against 0.01 M phosphate buffer.

The rate of formaldehyde reaction with DNA irradiated to form dimers or photolysed with hydrogen peroxide to make chain breaks was determined, and the rates of unwinding, I , were obtained. A value of v , the velocity of unwinding per defect, was then calculated from the known concentration of defects (either chain breaks or dimers). As Table 1 shows, the average value of v for breaks was 2.2 min^{-1} which agrees well with the value 2.4 to 2.5 min^{-1} obtained by Trifonov *et al.*⁴ for sheared DNA. This value is about an order of magnitude greater than that obtained for dimers, as indicated in Table 1. The difference does not, however, rule out the possibility proposed by Shafranovskaya *et al.*⁶ that the lower velocity of unwinding for dimers reflects clustering of dimers in the irradiated DNA.

To test their hypothesis we first treated irradiated DNA with ultraviolet-endonuclease, to convert dimer sites into chain breaks, and then treated it with formaldehyde. As Fig. 1 shows, the enzyme-treated sample reacted considerably faster

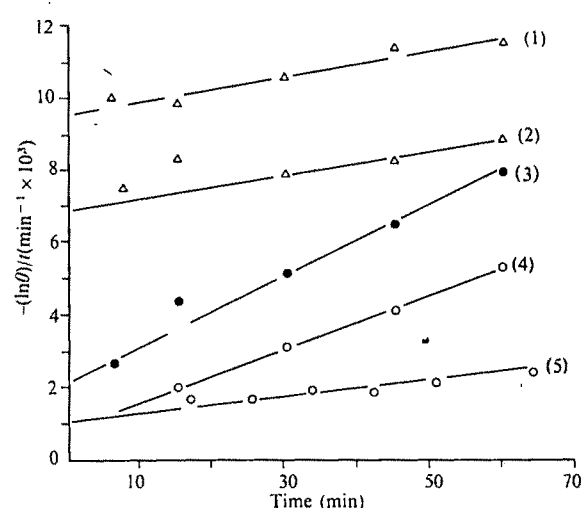


Fig. 1 Decrease in helicity, plotted as $(-\ln \theta)/t$ of ultraviolet-irradiated DNA exposed to formaldehyde for increasing lengths of time. Samples were treated with endonuclease in order to convert pyrimidine dimer sites into chain breaks. Quantitative analysis of the data is presented in Table 2. Curve (1) 800 J m⁻² with endonuclease; (2) 400 J m⁻² with endonuclease; (3) 800 J m⁻² without endonuclease; (4) no ultraviolet, with endonuclease; (5) no ultraviolet, no endonuclease.

than the untreated sample as judged by the large difference in their intercepts at $T=0$. (Enzyme treatment alone did not affect the intercept, although there was an increase in the slope of the curve.) As Table 2 indicates, the number of breaks per base pair made by the enzyme treatment was 3.3×10^{-3} , as compared with the initial concentration of pyrimidine dimers, 3.1×10^{-3} . This result implies that enzyme treatment made a break at the site of every dimer. It is calculated from the intercepts that the velocity of unwinding at chain break defects is seven to eight times faster than at pyrimidine dimer sites. This

Table 2 Kinetic Formaldehyde Analysis of Irradiated DNA Before and After Treatment with Ultraviolet Endonuclease

Ultraviolet fluence (J m ⁻²)	Endonuclease	M _w (daltons × 10 ⁻⁶)	Breaks per base pair (× 10 ³)	UT/T (× 10 ³)	TT/T (× 10 ³)	PyPy per base pair (× 10 ³)	I (min ⁻¹ × 10 ³)
400	—	4.39	—	4.8	7.6	3.1	<1
800	—	—	—	—	—	~6*	1.2
0	+	4.74	0	0	0	0	0
400	+	~0.36	3.4	4.1	8.1	3.1	5.5
800	+	—	~6*	—	—	—	8.7

* Since the samples receiving 800 J m⁻² contained no radioactive label, these values are estimations based on extrapolations from this and previous work.

difference suggests strongly that the result reported by Shafranovskaya *et al.*⁶ is not due to clustering.

Our conclusion—that chain breaks and dimers react with different velocities—is not surprising as the structure of DNA in the vicinity of a defect containing a single strand break would be expected to be less organised than that around a site containing a dimer, at which the sugar-phosphate backbone is still intact. Hence, formaldehyde reaction would be more likely to occur at a chain break than at a dimer.

A similar conclusion regarding base damage caused by ionising radiation was reached by Poverennyi *et al.*¹³, who observed that gamma-irradiated DNA reacted at the same rate with formaldehyde as DNase-treated DNA containing the same number of single strand breaks. Since gamma-irradiated DNA contains 4.5 times as many damaged bases (as determined by the loss of absorbance) as chain breaks, they concluded that base damage makes a negligible contribution to the reaction of gamma-irradiated DNA with formaldehyde.

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Effects of Low-dose X-irradiation on Chromosomal Non-disjunction in Aged Mice

THE factors responsible for the increasing incidence of offspring with Down's syndrome in older women are a continuing subject of debate. The demonstration of a greater frequency of aneuploid embryos in old mice^{1,2} may open the way for further experimental studies of the causes of some types of chromosomally abnormal conceptuses.

Clinical evidence suggests that the effect of maternal X-irradiation on the incidence of mongoloid children is a relatively small one³. Yamamoto *et al.*⁴ have presented results which, they claim, demonstrate that the bivalents of aged mouse oocytes are more susceptible to environmental injury than those of young oocytes. There is a decline in chiasma frequency and a corresponding increase in univalent frequency with age in mouse oocytes at diakinesis^{5,6}. Yamamoto *et al.*⁴ suggest that a deficiency of chiasmata could have predisposed aged oocytes to chromosomal damage during the long dictyate stage of meiosis. They argue from their results that, whereas

there was no significant effect of low-dose X-irradiation on the ova of young mice, there were significantly more chromosomally abnormal ova produced in old mice compared to young mice after irradiation. They emphasise the further increase in aneuploidy in old treated mice compared to old controls ($P=0.0337$ and 0.0600 for one and two-tailed tests, respectively). From all this they deduce (wrongly in our opinion) that the age of the mouse significantly affects the susceptibility of oocytes to irradiation damage. It should perhaps be mentioned that in comparing such small incidences, relatively large samples are required if significant differences are to be detected.

Table 1 Effect of low-dose X-irradiation on chromosome complement of 10.5-d-old mouse fetuses

Group	Total No. fetuses	No. aneuploid fetuses	% aneuploid fetuses (P)
Young non-irradiated	149	2	1.34
Young irradiated	111	4	3.60
Old non-irradiated	156	10	6.41
Old irradiated	43	7	16.28

* From the results of Yamamoto *et al.*⁴.

We have re-examined the data of Yamamoto *et al.*⁴ (Table 1) for evidence of interaction between age and X-irradiation. The differential effect of irradiation on ova from young and old mice was tested by comparing the appropriate function of the percentages with its standard error⁷. The value obtained was $(16.28 - 6.41 - 3.60 + 1.34) = 7.61$, with a standard error of 6.29, certainly a non-significant deviation from zero. This finding was confirmed by partitioning the three degrees of freedom of the contingency table into an age, irradiation and interactive effect. The latter comparison was found to be non-significant.

Both these statistical procedures, aimed at testing the conclusions of Yamamoto *et al.*, use linear differences in the percentages, which at such low incidences could prove quite misleading. A more meaningful method would be to examine the proportionate increases apparently due to irradiation, resulting in this example in the very similar ratios of $16.28/6.41 = 2.54$, and $3.60/1.34 = 2.69$ for old and young mice respectively. We have concluded therefore that these results do not show evidence of an interrelation of maternal age and radiation treatment.

Aneuploid embryos found in this study included uniform monosomics and trisomics as well as mosaics. Uniform aneuploids probably arise through chromosomal non-disjunction at meiosis I or, less likely, meiosis II, whereas mosaicism is produced by two cell lines developing in a single zygote. In the analysis of Yamamoto *et al.*⁴ these anomalies were grouped together, a fact which makes their conclusions even more questionable. If the mosaic embryos are excluded the incidence of aneuploid embryos found in their data is still higher in old (2.5%) compared to young (0) non-irradiated females ($P=0.0672$). A closer examination of the results of Yamamoto *et al.*⁴ strongly suggests that the data from their earlier published results¹ have been used as controls for the current irradiation studies. Such a procedure seems highly questionable in view of possible differences in environmental conditions.

In view of these objections we consider that the differential effect of X-irradiation on young and old oocytes to be not proven.

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New selective Giemsa technique for human chromosomes, C_a staining

AFTER the introduction of the quinacrine fluorescence method¹, several Giemsa staining techniques have been developed for karyotype analysis of human chromosomes. Pardue and Gall², originally noticed a denser staining of centromeric regions of chromosomes after *in situ* hybridisation of mouse chromosome preparations with mouse satellite DNA followed by Giemsa staining. This initial approach was modified by Arrighi and Hsu³ who omitted DNA hybridisation. Later, Sumner *et al.*⁴ left out treatment with RNase and HCl as well. Finally, McKenzie and Lubs⁵ treated chromosomes with HCl and 2×SSC only. These modified Giemsa procedures all produce densely stained regions of one or both chromosome arms close to the centromere. These C-band procedures also stain the secondary constriction of chromosome 1, 9 and 16, as well as the distal part of the Y chromosome. Satellites that stain brightly by Q-band techniques are also revealed by these methods.

Recent improvements of staining procedures have allowed an identification of particular chromosome areas. Among others Gagné and Laberge⁷ and Bobrow *et al.*⁸ have developed different techniques that selectively stain the secondary constriction of chromosome number 9.

Here I report a new banding technique which reveals two identical dots at the place of the centromere, one on each chromatid. The dots appear to have the same size on all forty-six human chromosomes (Fig. 1). This pattern may be termed C_a, for centromeric dots. The dots give the appearance of very densely stained and sharply delineated spheres. The

uniform size of the dots may suggest that they represent organelles associated with the spindle fibres. Prometaphase chromosomes tend to show a very narrow band instead of the separate dots, perhaps indicating that the centromere has not yet divided at this stage. The technique is specific for the centromeric regions, in contrast to other C-band treatments, that also give staining of the secondary constriction of chromosome 1, 9 and 16, the distal part of the Y chromosome, as well as satellites.

The procedure was as follows: human metaphase preparations were obtained by culturing heparinised whole blood, 0.2 ml per 3 ml 30% human inactivated AB-serum in Earle (MEM) medium. The culture was collected after 72 h and treated with colcemid (in a final concentration of 0.2 µg ml⁻¹) during the last 2 h. Hypotonic treatment followed, with 0.075 M KCl (5 min) at room temperature. The cells were fixated three times in methanol: acetic acid. The first time, the ratio of methanol: acetic acid was 9:1; the second time, 5:1, and the third time, 3:1. The cells were spread directly by a Carlsberg pipette on a wet glass surface at 13° C and air dried. Slides which had been stored for 1 week at room temperature were incubated in Earle's BSS medium (pH 8.5–9.0) at 85° C for 45 min. The slides were then stained in 1/25 Giemsa (Merck) in a 1/300 M buffered phosphate (pH 6.5) for 10 min. It seems very important to standardise the production procedure for chromosome preparation before heat treatment for C_a bands to be produced routinely. The culturing, hypotonic, and fixation treatment, as well as the age of the slide after preparation, do influence the banding pattern. The same parameters have also been found important in the case of the G, C and R-banding techniques⁹.

The physicochemical basis of this staining reaction is not yet understood. But it is known that the highly repetitive DNA found in areas of constitutive heterochromatin can be divided into various DNA families, differing in base composition, repetitiveness, and so on¹⁰. C_a bands possibly represent a specific DNA-protein complex which has been preserved after heat and alkali treatment.

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Relationship between DNA content of polytene chromosome bands and heterochromatization in *Drosophila*

THE variegated position effect is associated with chromosomal rearrangements involving heterochromatin (reviewed in ref. 1). In *Drosophila melanogaster*, cytological studies of the salivary gland chromosomes of strains carrying such rearrangements show that in some cells, the chromosome regions adjacent to broken heterochromatin lose their char-

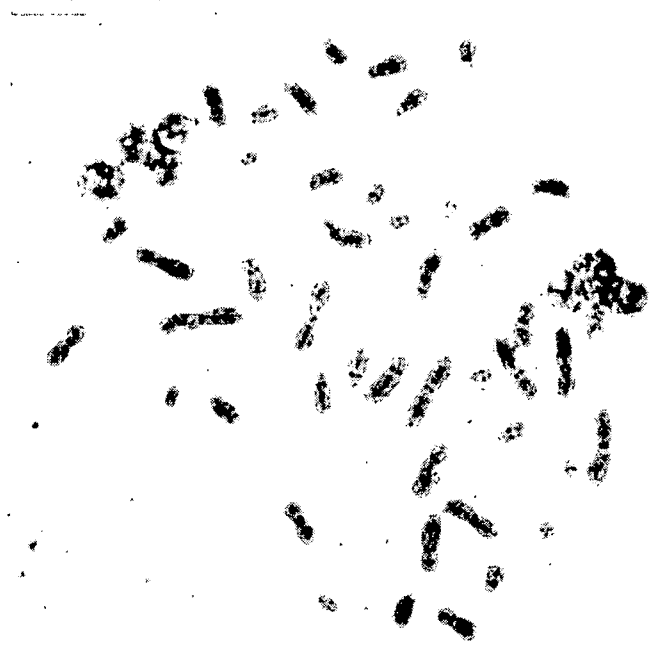


Fig. 1 Human metaphase chromosomes (46, XY). C_a bands. Only the centromeric regions are stained. They contain two identical dots at the centromere, one on each chromatid.

TABLE 1 Feulgen-DNA contents of chromosome regions in two strains of *D. melanogaster*

Region(s)	Single inversion strain*	Double inversion strain*	Diff. of means \pm s.e.	P
7E1-2	$M \pm$ s.e. 55.816 ± 5.594	$M \pm$ s.e. 58.075 ± 1.984	2.259 ± 5.935	n.s.
10A1-2	51.991 ± 6.703	53.625 ± 1.796	1.634 ± 6.939	n.s.
10D1-2	28.741 ± 3.020	23.941 ± 2.124	4.800 ± 3.692	n.s.
	$\bar{R} \pm$ s.e.	$\bar{R} \pm$ s.e.	t_{22}^\dagger	P
10D/7E	0.525 ± 0.032	0.414 ± 0.034	3.476	<0.01
10D/10A	0.574 ± 0.037	0.449 ± 0.040	2.315	<0.05
10A/7E	0.925 ± 0.044	0.927 ± 0.025	0.040	n.s.
	r	r	t‡	P
10D and 7E	0.814	0.297	1.805	n.s.
10D and 10A	0.874	0.269	2.332	<0.05
10A and 7E	0.877	0.660	1.138	n.s.

Salivary gland squashes from female white prepupae cultured at 14°C were stained by the Feulgen method. Using a Vickers M85 scanning microdensitometer, the integrated absorbance at 570 nm of the selected chromosome regions was measured in six non-heterochromatised nuclei in each of four preparations, two from each genotype. A $\times 100$ Planapochromatic objective of NA 1.3 gave a flying spot of diameter 0.47 μ m in the specimen plane, and 4% glare was offset electronically. Measured areas were precisely demarcated with a variable rectangular masking frame, and the apparent absorbance of an identical area of background, set to 90% transmission, was subtracted from each experimental reading. Each region was measured six times and the mean recorded.

M: mean Feulgen-DNA content of region in relative units.

\bar{R} : mean of ratios obtained by dividing Feulgen-DNA contents of region by that of reference band in same nucleus. *r*: correlation coefficient of Feulgen-DNA contents of two regions. For each strain, *n* = 12.

* for genotypes, see text.

† $t[(22)0.05] = 2.074$

‡ $t[(\infty)0.05] = 1.960$

acteristic banded appearance and become heterochromatised. The extent of this morphological change varies from cell to cell, but it has been noted that heterochromatisation seems to terminate preferentially just short of relatively large deeply staining bands^{2,3}. This observation might be due to the fact that such bands are more readily visible, and hence more likely to be scored as euchromatin than smaller bands. The involvement of other factors is however suggested by the observation³ that heterochromatisation associated with a given inversion in two genetic backgrounds terminates with different frequencies adjacent to certain bands which were morphologically similar in the two strains. A factor which, it has been suggested⁴, may affect the progress of heterochromatisation is a prior change in the nucleic acid content of affected regions. Early measurements of nucleic acid content using ultraviolet photographic microdensitometry of whole bands⁴ or spot estimations of optical density⁵ yielded equivocal results. We decided to use scanning microdensitometry to investigate the possibility that the differences in extent of heterochromatisation observed in our strains might be related to the DNA content of the bands concerned.

The Feulgen-DNA content of one affected band, 10D1-2, (terminology according to Bridges⁶) was measured in the single and double inversion strains, *m^x;+* and *m^x;Rev^p*. To correct for variations in staining and polyteny, the Feulgen-DNA contents of bands 10A1-2 and 7E1-2 were used as internal standards: 10A1-2 lies within the region subject to position effect but shows the same response to heterochromatisation in both strains, and 7E1-2 is beyond the region of potential heterochromatisation.

The results are summarised in Table 1. Mean uncorrected Feulgen-DNA contents of bands did not differ significantly between the strains, although in the case of band 10D1-2 the decrease in the double inversion strain approached statistical significance. When standardised against either of the reference bands, the mean value for 10D1-2 was significantly smaller in the double than in the single inversion strain. These results were confirmed by analysis of covariance (10D1-2 and 7E1-2: $F(1/22) = 4.55$, $P < 0.05$; 10D1-2 and 10A1-2: $F(1/22) = 4.63$, $P < 0.05$). In the single inversion strain there was a significant correlation between the Feulgen-DNA contents of 10D1-2 and each reference band, while in both strains there was significant correlation

between the two reference bands. Thus in the double inversion strain band 10D1-2 showed a relative, and possibly an absolute, decrease in Feulgen-DNA content and also exhibited less related variation than in the single inversion strain. Measurements were also made of band 50A1-2, a euchromatic band within the *Rev^p* inversion; of band 80A1-2, a relatively well defined heterochromatic band at the base of chromosome 3L; and of part of the chromocentral region which corresponded morphologically as closely as possible in each nucleus. No strain differences were found at these sites.

Band 10D1-2 therefore seems to be unusual not only in its relation to termination of heterochromatisation but also in its Feulgen-DNA content. The coincidence of these peculiarities suggests that the apparent irregularity in progression of heterochromatisation along the chromosome is not merely an observational artefact, but rather a genuine phenomenon related to some biological property of the bands concerned. Since the DNA content of a prepupal chromosome band presumably reflects the replication behaviour of the band during embryonic and larval development, a causal connection between heterochromatisation and DNA replication seems plausible. It should be noted that the chromosomes selected for measurement were not heterochromatised. Our results indicate that an additional rearrangement not only affects variegation and heterochromatisation³, but also influences chromosome bands in (potentially heterochromatised) regions which are morphologically euchromatic.

It is tempting to speculate that a break in heterochromatin leads to a change in replication behaviour of some euchromatic bands in its vicinity, and that the progression of heterochromatisation, when it occurs, is affected by such changes.

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Light-regulated guanosine 3', 5'-monophosphate phosphodiesterase of bovine retina

RETINAL rod outer segment (ROS) adenylyl cyclase activity seems to be light sensitive, implicating adenosine 3',5'-monophosphate (cyclic AMP) in photoreceptor function^{1,2}. Guanosine 3',5'-monophosphate (cyclic GMP) could also play a role in photoreceptor function since the synthetic (guanylyl cyclase)³ and degradative (cyclic GMP phosphodiesterase)⁴ enzymes are highly active in purified ROS. In particular the specific activity of guanylyl cyclase is higher in bovine photoreceptor structures than in any other tissue studied. This activity, however, is not influenced by illumination³.

To assess cyclic GMP formation in a more physiological system, we followed the formation of ³H-cyclic GMP from ³H-hypoxanthine in isolated intact cattle retina. In the presence of a phosphodiesterase inhibitor, no differences could be detected between illuminated and dark-adapted retinæ. By contrast, without the inhibitor, a much lower ³H-incorporation was observed after preillumination (Table 1).

TABLE 1 Formation of ³H-guanosine 3',5'-monophosphate from ³H-hypoxanthine by bovine retina

	c.p.m.g ⁻¹ retina	
	With SQ 20,009	Without SQ 20,009
Exp. 1		
Dark	4,040	3,490
Light	3,420	1,000
Exp. 2		
Dark	6,520	4,580
Light	6,280	740

Young bovine eyes were obtained from a slaughterhouse and dark-adapted for 2 h at 0° C. Subsequent operations were carried out in dim red light. The retinæ were preincubated for 30 min at 37° C in Krebs-Ringer-bicarbonate buffer (pH 7.4, continuously gassed with 95% O₂ - 5% CO₂). After prelabelling (60 min at 37° C) with 2.5 µM ³H-hypoxanthine (5 µCi per retina), the retinæ were washed by five successive transfers into 100 ml beakers and then distributed one by one into 10 ml Erlenmeyer flasks containing 3.5 ml Krebs-Ringer-bicarbonate buffer with or without 0.3 mM cyclic nucleotide phosphodiesterase inhibitor SQ 20,009 [1-ethyl-4-(isopropylidenehydrazino)-1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid ethylester]. The retinæ were then either left in the dark (0° C), or illuminated at 0° C in 5 ms (unfiltered flash light having a maximum energy of 30 J, 10 cm above the incubation vessel). The final incubation (10 min at 37° C) was started immediately. The retinæ were collected by rapid filtration and homogenised in 8% trichloroacetic acid (TCA). TCA was extracted by ether and the ³H-cyclic GMP separated from other nucleotides by successive chromatography on aluminum oxide (1.5 g Al₂O₃, Woelm W200 neutral) equilibrated with 0.06 M Tris-Cl buffer, pH 7.5) and Dowex 1 × 8 (formate, 0.5 × 4.5 cm) columns. The Al₂O₃ columns were washed with 2 ml 0.06 M Tris-Cl buffer, pH 7.5, and cyclic GMP eluted with 2 ml 0.6 M Tris-Cl buffer, which was passed through the Dowex columns. After a wash with 6.5 ml 0.5 N formic acid the cyclic GMP was eluted with 3 ml 8 N formic acid. Unlabelled cyclic GMP had been added to the TCA extracts to correct for recovery (51-64%). If the Dowex eluate was treated with beef heart phosphodiesterase (Sigma), less than 5 % of the radioactivity originally present could be detected after a subsequent chromatography on Dowex 1 × 8. No radioactivity from added ¹⁴C-cyclic AMP was found in the cyclic GMP fraction.

The results are expressed as c.p.m. in ³H-cyclic GMP per g fresh weight. Values are the mean for two retinæ incubated in parallel, the single determinations differed from the mean less than ±12 %.

Although these values, uncorrected for the specific activity of GTP, by no means measured exactly the synthesis of cyclic GMP, they indicate that retinal cyclic GMP levels could be regulated by light-dependent changes in phosphodiesterase activity. In fact, the cyclic GMP phosphodiesterase activity of retinal homogenates incubated in the light was more than three times that of homogenates assayed in the dark (Table 2). But in accord with previous studies,¹ cyclic nucleotide phosphodiesterase activity of purified ROS was not light sensitive. As expected, illumination was without effect on brain phosphodiesterase.

These results might suggest that a phosphodiesterase localised elsewhere than in the ROS could be influenced by light. But 84% of total retinal cyclic GMP hydrolysing activity is associated with the outer segments prepared and tested in the dark (unpublished).

With cyclic AMP as substrate, a much smaller light activation was seen (Table 2). This result is in keeping with the observation that cyclic GMP is the preferred substrate for ROS cyclic nucleotide phosphodiesterase, whereas an activity with a low *K_m* for cyclic AMP is localised in other retinal structures^{4,6}.

TABLE 2 Effect of light on retina cyclic nucleotide phosphodiesterase

Preparation	nmol GMP formed/mg protein/min	
	Light	Dark
Bovine retina homogenate	29.0 ± 1.6*	8.5 ± 0.8*
Bovine rod outer segments	157	154
Rat brain homogenate	28.3	26.7
	nmol AMP formed/mg protein/min	
	Light	Dark
Bovine retina homogenate	9.3 ± 0.6†	6.3 ± 0.8†

Retinæ from dark-adapted young bovine eyes (1-3 h at 0° C) were homogenised by hand in 5 volumes (w/v) 0.25 M sucrose-10 mM Tris-HCl buffer, pH 7.5, and aliquots (600-800 µg protein) assayed⁵ for cyclic nucleotide phosphodiesterase activity with either 1 mM cyclic GMP or 1 mM cyclic AMP in diffuse room light or in complete darkness (5 min at 37° C). To exclude the possibility that the reaction might go further than to the corresponding nucleosides, ³H-GMP and ³H-AMP were incubated with retina homogenates. Their recoveries were not influenced by light and virtually identical with those of the ³H-nucleosides added at the end of the incubation (70-76% for guanosine and GMP, 64-66% for adenosine and AMP).

* Mean value for nine experiments ± s.e.m. (*P* < 0.01).

† Mean value for four experiments ± s.e.m. (0.05 > *P* > 0.01).

The light activation could be mimicked by adding bleached ROS preparation devoid of phosphodiesterase activity and shown to contain no protein other than opsin, by gel electrophoresis⁷. ROS, prepared as described elsewhere⁸, were extracted with 0.1% sodium dodecyl sulphate (SDS), which was removed by repeated washing. Such preparations, left in the dark or illuminated until 65% bleaching had occurred, were added to the cyclic GMP phosphodiesterase assay. There was no effect on retina phosphodiesterase activity determined in the light (Table 3). Addition of the bleached preparation, however, increased the dark activity almost up to the activity determined in the light. Unbleached rhodopsin had also a small stimulating effect on the dark activity, possibly due to the presence of some bleached rhodopsin. The rhodopsin preparations contain membrane-bound lipids including retinal. But all-trans retinal, which is released during bleaching, had no effect on cyclic GMP phosphodiesterase activity measured in light or dark if added in concentrations equivalent to the amount present in the rhodopsin preparations. Traces of bound SDS could have a stimulatory effect. The SDS should, however, exert its effect on both light and dark activity. Furthermore, brain and liver phosphodies-

TABLE 3 Effect of adding preparations of photoreceptor membranes on retinal cyclic GMP phosphodiesterase activity

Addition	nmol GMP formed/mg protein/min	
	Light	Dark
Dark-adapted ROS preparation	33.9	14.5
Bleached ROS preparation	33.8	32.6
Control	34.3	8.7

ROS purified as described previously,⁸ were extracted twice with 0.1 % SDS—10 mM Tris-HCl buffer (pH 7.5). The insoluble material was washed first six times with 0.25 M sucrose—10 mM Tris-HCl (pH 7.5) and finally taken up in the same buffer (1 mg protein ml⁻¹). One part of the preparation was illuminated for 5 min at 0° C (33 W tungsten lamp, distance 4 cm), the rest left in the dark. Spectrophotometric estimations⁹ showed that 65% of the rhodopsin was bleached in the light-exposed preparation and less than 10% in the dark-adapted one. Aliquots containing 30 µg protein were added to samples of a retina homogenate (650 µg protein) and cyclic GMP phosphodiesterase activity determined in diffuse room light or in complete darkness. Buffer only was added to the controls.

terases, though stimulated by low SDS, were affected neither by dark- nor by light-adapted preparations.

Cyclic nucleotide phosphodiesterase inhibitors have been shown to have a profound effect on frog¹⁰ and *Limulus*¹ photoreceptor potentials. Our data show that regulation of ROS phosphodiesterase activities could be also physiologically important, since retinal cyclic GMP phosphodiesterase activity was strongly influenced by light. The light effect was most probably dependent on the bleaching state of rhodopsin, since it could be mimicked by adding light-exposed ROS preparations devoid of phosphodiesterase activity, where all components soluble in buffer and low concentrations of SDS had been eliminated. The light-provoked decrease of ³H-cyclic GMP levels in incubated retinac, not seen after phosphodiesterase inhibition, suggests that light-dependent changes in cyclic GMP phosphodiesterase activity occur also in intact retinac.

Cyclic nucleotide phosphodiesterase of taste receptors has been shown to be activated by bitter taste stimuli¹¹. Activation of phosphodiesterase activity by the appropriate stimuli might be a general phenomenon for the function of sensory receptors.

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Correlations between (Na + K)-ATPase, Ca-ATPase and cellular potassium concentration in cattle red cells

THE existence in erythrocytes of active, ATP-dependent Na-K exchange on one hand and of active, ATP-dependent outward transport of Ca¹⁻⁵ on the other raises the question whether two independent or one common system account for these functions. Inhibition of the Na-K system by Ca, although easily demonstrated, does not lend particularly strong support to the hypothesis of a common pathway for Na and Ca in active transport, because full activation of the Ca system⁶⁻⁹ is achieved by lower Ca concentrations than those required to inhibit Na+K-ATPase⁸ and because Epstein and Whittam¹⁰ have pointed out that the effect is due to competition between Ca-ATP and Mg-ATP for the substrate site and that competition between Ca and Na for the transport site is unlikely. The Na-K system is inhibited by cardiac glycosides whereas Ca transport and Ca-activated ATPase are not^{2,3,11} which seems to exclude a common site, but not necessarily a common protein. If alkali cation and Ca movements were mediated by the same membrane protein independent measurements of Na+K-activated ATPase and Ca-stimulated ATPase activity should positively correlate. K concentration in cattle red cells varies widely between animals^{12,15}, there is agreement as to the ability of these cells to pump Na and K¹³⁻¹⁷, and indications exist that different intensity of pumping accounts for the different K (and Na) content of the cells¹²⁻¹⁴. Cattle cells, therefore, seem suitable objects to study phenomena which might correlate with Na+K-ATPase.

Blood (100 ml) from healthy adult (above 2-yr-old) Simmental and Swiss Brown cows was prevented from clotting by adding approximately 17 U ml⁻¹ heparin (USP) and brought to the laboratory within a few hours. The cells were washed four times with a solution of (mM) 120 choline-Cl, 40 Tris-Cl, pH 7.4 in the cold. Na-free solution was chosen to avoid any possible Na-Ca exchange during washing. White cells were discarded. K in washed and packed cells was measured by flame photometry in the haemolysate (0.1 ml cells + 7.9 ml H₂O). For the measurement of cellular Ca the cells were diluted 1:4 with water, the mixture deproteinised with an equal volume of trichloroacetic acid (10%) and the supernatant made up to 50 mM LaCl₃ in another 1:2 dilution step to prevent interference of phosphate. Standards of CaCl₂ were made up in the same water and LaCl₃-solution and the samples were measured with an EEL atomic absorption flame photometer with scale expansion unit and recorder. Results were corrected for Ca contamination of trichloroacetic acid. The choline-washed cells were further washed twice with a solution of (mM) 150 NaCl, 5 KCl, 1 MgCl₂ and membranes were prepared from them according to Wolf⁷ as described earlier⁶. The membranes were kept frozen until used (36 h at most). Na+K-ATPase was measured as the ouabain-inhibitable fraction in a medium of (mM) 100 NaCl, 10 KCl, 4 MgCl₂, 0.5 tris-EGTA, 30 imidazole-Cl, pH 7.0 (at 37°C), 2 Mg-ATP, with or without ouabain 10⁻⁴ g ml⁻¹. Ca-ATPase was assayed in the following medium: (mM) 70 choline-Cl, 4 MgCl₂, 30 imidazole-Cl, pH 7.0 (at 37°C), 0.5 Ca-EGTA buffer (of 10⁻³M Ca²⁺ concentration) or 0.5 Tris-EGTA, 2 Mg-ATP and 10⁻⁴ g ml⁻¹ ouabain. The samples contained on average 1.47 mg protein in 2.5 ml, incubation time was 90 min and temperature 37°C. In six experiments Ca-ATPase was measured in the absence of Mg, Mg-ATP being replaced by Na-ATP. All samples were run in duplicates. P_i liberated and protein were measured as before⁶. Since high K cells are rare^{12,13} many animals were sampled for K content of cells and twenty-one specimens were selected such as to have all K concentrations about equally represented.

It was found that cellular Ca concentration is far below the Ca^{2+} concentration in blood plasma, much as was reported for human red cells^{18,19}. The average from thirteen

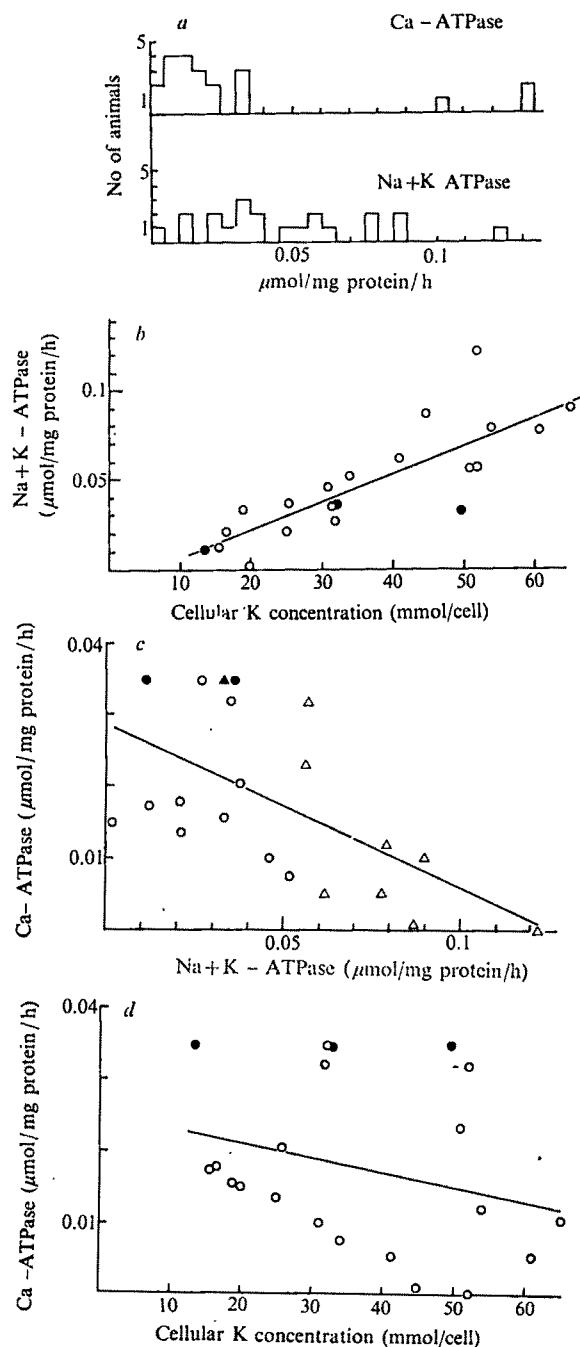


Fig. 1 Cellular K concentration, Na+K-ATPase and Ca-ATPase activity in erythrocytes of twenty-one cows. Ca-ATPase: difference between sample with 10^{-5}M Ca^{2+} and sample with 0.5 mM EGTA (medium: choline 70, imidazole 30, Mg-ATP 2, Mg 4, Cl 108 mM, ouabain 10^{-5} g/ml). Na+K-ATPase: difference between sample without and with ouabain 10^{-4} g/ml (medium: Na 100, K 10, imidazole 30, Mg-ATP 2, Mg 4, Cl 148 mM). pH 7.0, 37°C , 90 min. Cellular K concentration measured in fresh cells, washed with choline-Cl 120 mM, tris-Cl 40 mM, pH 7.4. a, b, c, d: Same data plotted differently. a: Frequency distribution of Na+K-ATPase does not peak, because animals were selected in order to have all K concentrations equally represented (compare with b). Notice three outliers in the Ca-ATPase values from the same animals. b, c, d: Each point represents 1 animal. b, $r = 0.819$, $P < 0.001$; c, $r = -0.588$, $P < 0.01$; d, $r = -0.272$, $P > 0.05$. Solid symbols = Ca-ATPase outliers (Ca-ATPase values corrected as described in text). c, Triangles = animals with K concentration in cells $> 40\text{ mmol l}^{-1}$ cells. Calculated regression lines and r values include all points shown.

animals was $0.024 \pm 0.003\text{ mmol l}^{-1}\text{ cells}$ (\pm s.e.m.). This is not due to the washing procedure since unwashed cells gave values similar to what was predicted on the assumption of virtually Ca free cells contaminated by 6–8% blood plasma. No correlation between cellular Ca content and Ca-ATPase activity of the corresponding membranes was found, which is not surprising, as it is well known that in human red cells total Ca is largely accounted for by Ca associated with the membrane and does not reflect intracellular Ca concentration^{18,19}.

Figure 1a shows the frequency distribution of the Ca-ATPase in twenty-one animals. The average, omitting the three outliers, was $0.0151 \pm 0.0024\text{ }\mu\text{mol per mg protein per h}$ (\pm s.e.m.) which is about 1/50 of what is found in human red cells under similar conditions. The outliers were 9.6 standard deviations or more away from the mean and were, therefore, omitted from the statistical evaluation or replaced by the highest value of the rest of the distribution (0.0348) as shown in Fig. 1 by solid symbols (C. P. Windsor, quoted in ref. 20.) Preliminary experiments indicated that the dissociation constant K_a of the enzyme for Ca^{2+} is of the order of 10^{-6}M . There is some Ca-stimulated activity in the absence of Mg. In six experiments this amounted to 14.3% of the total Ca-stimulated activity in the presence of Mg. This activity was not separated from the total in the statistical treatment.

Figure 1b shows that a close positive correlation exists between Na+K-ATPase activity and cellular K content. The calculated regression line extrapolates to the reasonable K concentration of 6 mmol l^{-1} cells at zero Na+K-ATPase activity. This is according to expectation and agrees with the accepted view that the Na-K pump is a major factor controlling cellular K concentration.

The main finding is illustrated in Fig. 1c. Ca-ATPase activity and Na+ATPase activity correlate negatively ($P < 0.05$ when the outliers for Ca-ATPase are omitted, $P < 0.01$ when they are reduced to $0.0348\text{ }\mu\text{mol mg}^{-1}\text{ h}^{-1}$ in a two-tailed test). Triangles represent samples which can be attributed with a high degree of probability to the class of animals with genetically determined high K cells in our breeds^{12,13}. Within this group the correlation coefficient (r) is -0.807 (with $P = 0.009$) when the outlier is reduced to 0.0348.

Figure 1d demonstrates that no significant correlation was found between Ca-ATPase activity and cellular K content. But when the partial correlation coefficient ($r_{D.bc}$) is calculated (eliminating the effect of the correlations b and c of Fig. 1), a positive correlation is revealed with $r_{D.bc} = 0.450$ and $z\sqrt{N-4} = 1.953$. For a two-tailed test

$$z\sqrt{N-4} = \frac{1}{2} \cdot \ln[(1+r_{D.bc})/(1-r_{D.bc})] \cdot \sqrt{N-4} > 1.96 \text{ for } P < 0.05$$

with N = number of points. This means that the positive correlation is just significant. Thus it seems not unlikely that the negative correlation between Na+K-ATPase and Ca-ATPase masks a positive correlation between Ca-ATPase and K content of the cells.

The negative correlation between Ca-ATPase and Na+K-ATPase makes the hypothesis of a single system for both improbable. Competition between alkali cations and Ca can be ruled out because Na+K-ATPase was assayed in the absence of Ca and Ca-ATPase was measured in the absence of Na and K (except for approximately 0.2 mM stemming from the membrane preparation) and in the presence of ouabain. No interpretation with respect to cause and effect can be offered for the negative correlation at present, but several possibilities might be considered. (1) The Na-K pump requires traces of Ca inside the membrane and the Ca pump removes Ca more effectively from the critical region than EGTA alone does. (2) A fixed number of molecules of an individual protein are synthesised in the maturing cell

and an unknown factor determines whether any one of these behaves as a Ca- or Na-K-pump site. (3) In the synthesis of two distinct pump proteins some step is common for both and limiting and the final assembly into structurally different complexes determines the functional use of the common part. (4) An unknown factor is inhibitory for one and stimulatory for the other of two distinct pumps.

The positive correlation between Ca-ATPase and K content of fresh cells might mean that a low intra-cellular Ca concentration facilitates maintenance of high intracellular K concentration. It is well known^{5,21-25} that a small increase in cellular Ca concentration renders human red cells leaky to K. The present finding might reflect this effect of intracellular Ca* and, therefore, could substantiate the view expressed by Gardos²¹ as early as 1958 that cellular Ca content might influence cellular K concentration not only under abnormal experimental conditions but also *in vivo*.

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* *Note added in proof:* This interpretation is doubtful, as a recent report (Jenkins and Lew, *J. Physiol., Lond.*, **234**, 41 (1973)) demonstrates the absence of the Gardos-effect in ruminant red cells.

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Molecular weight of a D-glucose and L-histidine-binding protein from intestinal brush borders

We describe here a sugar and amino acid transport protein that has been isolated from a cell for the first time. It is

a homogeneous protein subunit from the mucosal brush border of hamster jejunum that is involved in the Na⁺-dependent binding of actively transported D-glucose and L-histidine. Its molecular weight was estimated to be 59,000 by Sephadex G-75 gel filtration¹, 56,000 by sodium dodecyl sulphate-acrylamide gel electrophoresis^{2,3}, and 50,934 by sedimentation velocity analysis^{4,5}. The presence of both D-glucose and L-histidine-binding sites on the same protein with a molecular weight of approximately 55,000 may account for some of the observed competitive inhibitory effects between active intestinal sugar and amino acid transport that have been reported⁶.

Our work is based on the results of many studies which have indicated that active transport of sugars and amino acids by the small intestine occurs within the brush border of the mucosal cell and requires sodium ions⁷⁻¹⁰. In an attempt to describe these absorptive mechanisms, we have shown that actively transported sugars and amino acids are preferentially bound to isolated brush borders^{11,12} and that both D-glucose and L-histidine require Na⁺ to bind to separate sites in a filamentous fraction of disrupted brush borders^{13,14}. Since the brush border is a digestive, as well as an absorptive organelle¹⁵, we have postulated that its core filaments act as a conduit for the Na⁺-dependent active transport of all monosaccharides and all amino acids which are primarily obtained from the hydrolysis of disaccharides and polypeptides on the plasma membrane facing the lumen¹⁴. The purpose of the study reported here was to solubilise the brush border fraction involved in the Na⁺-dependent binding of D-glucose and L-histidine, and determine its molecular weight.

Mucosal brush border filaments from hamster jejunum were prepared^{13,14} and labelled with ¹⁴C by incubation at 37° C for 30 min in either 0.1 μM of uniformly labelled ¹⁴C-D-glucose (288 mCi mmol⁻¹) or 0.5 μM of uniformly labelled ¹⁴C-L-histidine (240 mCi mmol⁻¹) in 25 mM Na₂HPO₄-NaH₂PO₄ buffer (pH 7.2), 10 mM MgCl₂, and 6 mM dithiothreitol (DTT). After the incubation period the suspension was centrifuged and the supernatant was removed. Then the filamentous precipitate was extracted with cold acetone. Most of the radioactivity remained in the insoluble precipitate indicating that the ¹⁴C-labelled compound was not bound to the lipid-soluble portion of the filaments. The acetone powder was solubilised by incubating the ¹⁴C-labelled material, at 25° C for approximately 12 h, in 1% SDS, 25 mM sodium phosphate buffer (pH 7.2), 10 mM MgCl₂, 6 mM DTT, and the same concentration of the ¹⁴C-labelled compound that was used in the binding experiment. Figure 1 illustrates a typical elution profile of this solution obtained by Sephadex G-75 gel filtration. In the experiment illustrated, ¹⁴C-D-glucose was bound within a sharp peak absorbing at 230 nm. Though there was some absorption before and after the peak it did not obtain any bound ¹⁴C-D-glucose. Free radioactive sugar appeared in the elution volume between 450 ml and approximately 550 ml. This was confirmed by control experiments in which only free ¹⁴C-D-glucose was placed on the column. Similar results were obtained when ¹⁴C-L-histidine was used as the binding compound. Although both ¹⁴C-D-glucose and ¹⁴C-L-histidine were bound within the same peak of absorbance, more ¹⁴C-D-glucose ($4.17 \pm 0.96 \times 10^{-8}$ mmol) than ¹⁴C-L-histidine ($1.95 \pm 0.07 \times 10^{-10}$ mmol) was bound per mg of protein as determined by four and three experiments, respectively. The amount of the ¹⁴C-labelled compound bound was calculated from its measured radioactivity and specific activity. Protein was determined by the Lowry *et al.* method using bovine crystalline serum albumin as standard¹⁶.

To verify that the isolated protein is the component involved in Na⁺-dependent binding of actively transported nutrients by the hamster jejunum, the denaturing SDS adhering to it had to be removed. This was accomplished by

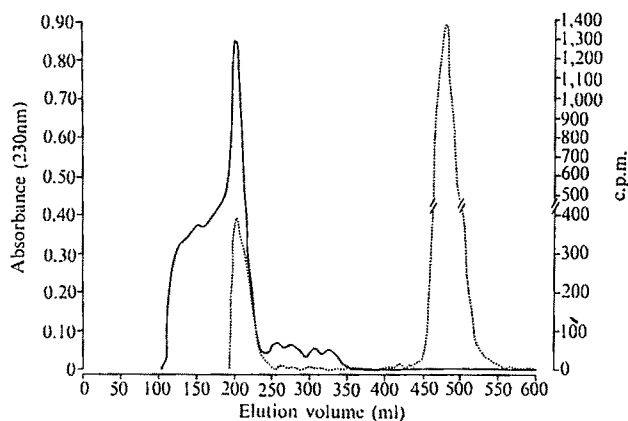


Fig. 1 Elution profile from gel filtration of acetone extracted, SDS-solubilised brush border filaments labelled with ^{14}C -D-glucose in the presence of Na^+ ions. Before the solubilised material was placed on a Sephadex G-75 gel bed in a 2.6×100 cm column, it was diluted with 25 mM of $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ buffer (pH 7.2) containing 10 mM MgCl_2 and 6 mM DTT to achieve a 0.1% SDS concentration, filtered with a $0.45 \mu\text{m}$ pore diameter filter, and centrifuged at 112,000 g for 2 h. The small amount of precipitate obtained after centrifugation was discarded. A 4 ml sample of the supernatant containing approximately 5 mg of protein were placed on the Sephadex G-75 gel filtration column eluted with 25 mM sodium phosphate buffer (pH 7.2) containing 0.1% SDS, 10 mM MgCl_2 , and 6 mM DTT. The void volume of the gel column was 140 ml. After the absorption of the eluant was read at 230 nm to detect peptide bonds, each 5 ml fraction was dried at 80°C in a vial. Then 15 ml of liquid scintillation fluid was added to each vial before it was counted in a Nuclear Chicago mark I liquid scintillation system. The peak in absorbance had bound $4.17 \pm 0.96 \times 10^{-8}(4)$ mmol of ^{14}C -D-glucose per mg of protein. An absorption spectrum of this peak was performed after it had been precipitated by 30% $(\text{NH}_4)_2\text{SO}_4$ and redissolved in 25 mM sodium phosphate buffer (pH 7.2). Absorption occurred at approximately 280 nm and 230 nm, demonstrating that the peak contained protein relatively free from other ultraviolet absorbing compounds. —, Absorbance; •••, D-U- ^{14}C -glucose.

dissolving the unlabelled, partially purified binding protein, obtained by precipitation with 30% $(\text{NH}_4)_2\text{SO}_4$, in 50 mM Tris-acetate buffer, pH 7.8, containing 6 mM DTT and 6 mM urea. This solution was passed through a SDS-removal column containing Dowex 1-X2¹⁷. The SDS-free effluent was then placed in the hollow bore fibres of Bio-Fiber 50 minibeaders obtained from Bio-Rad Laboratories to exchange 25 mM sodium, potassium or ammonium phosphate buffer, pH 7.2, for the Tris-acetate buffer and to remove urea. The buffer on the outside of the hollow fibres was replaced every 15 min for 1 h at 5°C . The last replacement contained $0.1 \mu\text{mol}$ of ^{14}C -D-glucose (uniformly labelled; 288 mCi mmol⁻¹). Diffusion of the ^{14}C -D-glucose across the fibre walls was then allowed to proceed for 1 h at 37°C . We had previously determined, in the absence of protein, that ^{14}C -D-glucose can reach a diffusion equilibrium under these conditions. After this incubation period, ^{14}C in 0.1 ml samples of the solutions inside and outside the hollow fibres were counted in a liquid scintillation system. The results from these studies demonstrated that ^{14}C -D-glucose was at a higher concentration within the hollow fibre compartment containing the binding protein only when Na^+ ions were present. When either K^+ or NH_4^+ were substituted for Na^+ in the phosphate buffer, ^{14}C -D-glucose was observed to be at the same concentration in both compartments. Therefore, the increase in ^{14}C -D-glucose within the protein compartment in the presence of Na^+ could only be attributed to the binding of this sugar to the isolated protein. The amount of sugar bound per mg of protein was similar to that observed in the absorbance peak from the Sephadex G-75 gel filtration column (Fig. 1).

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Narcotic Receptor Sites in Morphine-dependent Rats

THE biochemical basis of the development of tolerance for and dependence on narcotic analgesics has been the subject of much speculation and experiment¹⁻⁶. Theories which try to account for these closely related^{4,7} phenomena can be grouped into three main classes: (1) the body reacts to narcotic drugs by increasing the rate at which they are destroyed or neutralised (by antibodies, for example) so that a smaller proportion of the total dose reaches the brain receptors²; (2) processes which are directly affected by the narcotic analgesics are circumvented or otherwise accommodated through an indirect physiological adaptation⁶; (3) a direct adaptation takes place in which the number of receptor sites for the narcotics is greatly increased, or their affinities greatly reduced so that large amounts of drugs are needed to fill the available sites and thus effect a response⁵. We wish to present data which effectively rule out this third possibility as a major factor in narcotic drug dependence and tolerance. We show that neither the number, nor the binding affinity, nor the specificity of narcotic receptor sites⁸⁻¹⁰ is changed in the morphine-dependent (and therefore also tolerant⁷) rat brain when compared with that of the normal animal.

Osborne-Mendel rats, 150–200 g, from the NIH colony, were used, mostly males, although one set of experiments involved females, with equivalent results. Morphine dependence was induced by implanting pellets containing 75 mg of morphine base plus CM cellulose carrier and binders^{7,11}, subcutaneously near the midline of the back just behind the ears¹². Light ether anaesthesia was used. This procedure induces clear-cut tolerance to and dependence on morphine, which is maximal

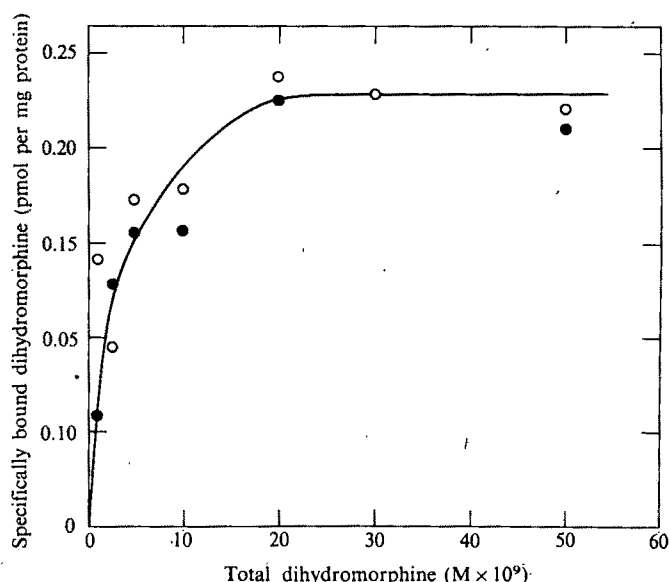


Fig. 1 The specific binding of ^3H -dihydromorphine to particulate fractions of rat brain homogenates prepared from control (○) and morphine-dependent (●) rats. The data are obtained by subtracting the amount of non-specifically bound ^3H -dihydromorphine, measured in the presence of 10^{-5} M morphine (a saturating amount) from the amount bound in the absence of unlabelled morphine. Each flask contained 0.5 mg of P_2 protein, 9 μmol Tris-HCl, pH 7.4, and ^3H -dihydromorphine (31,000 c.p.m. pmol^{-1}) in the amounts shown, in 1 ml of 0.32 M sucrose. The points shown are the average of two independent experiments each of which was performed with duplicate determinations.

after 72–96 h and declines slowly during the next few days¹². Spot checks on some animals 72 h after operation with an intraperitoneal injection of the antagonist naloxone (1 mg of the hydrochloride in 0.1 ml H_2O) showed that the animals carrying the morphine pellet went into almost immediate withdrawal^{12,13}, characterised by extreme hyperactivity, sensitivity to touch (manifest by squealing), wet dog shakes and diarrhoea. Control, sham operated, animals were unaffected by naloxone.

The animals were decapitated 72–84 h after implantation of the morphine pellet. Brains, without the cerebellum, were homogenized in 10 volumes of 0.32 M sucrose using twenty strokes of a loosely fitting Teflon-glass homogenizer. The homogenate was centrifuged at 1,000g for 10 min and the supernatant fraction was centrifuged at 10,000g for 10 min. The pellet (P_2)¹⁴ was suspended in the original volume of 0.32 M sucrose and diluted with 8 volumes of 0.32 M sucrose, 0.01 M Tris-HCl, pH 7.4, for assay. Brains of three to six rats were pooled after homogenisation in each of four independent experiments, all of which gave equivalent results.

Narcotic drug binding was studied with the aid of ^3H -dihydromorphine, specific activity 51.2 Ci mmol^{-1} (New England Nuclear Corp.). Brain homogenates or, more usually, the 10,000g particulate fractions P_2 (0.5 mg of protein in 900 μl of 0.32 M sucrose, 0.01 M Tris-HCl, pH 7.4), were incubated with ^3H -dihydromorphine in the presence and absence of unlabelled morphine (or other drug) at a concentration such that the specifically bound dihydromorphine was displaced by the unlabelled narcotics. After 10 min at 37° C, unbound radioactive material was removed by centrifugation at 19,000 r.p.m. for 10 min. The tube and surface of the pellet was washed with 1 ml of 0.32 M sucrose and the washed pellet, suspended in 1 ml of 1% Triton X-100, was assayed for radioactivity. All operations involving dihydromorphine were carried out in the dark or in a room illuminated with extremely dim, indirect daylight. At 10^{-9} M dihydromorphine, as in Figs. 2–4, approximately half the bound radioactivity was specifically bound in that it was displaced by low concentrations of narcotics but not by their inactive stereoisomers.

Residual morphine present in the P_2 fraction of the homogenate of the brains of morphine-treated animals was estimated after extraction by the method of Mulé¹⁵. The organic solvent extracts, after removal of the solvent, were dissolved in a small volume of methanol. Aliquots were assayed for morphine by measuring the amount of bound ^3H -dihydromorphine displaced in our standard binding assay compared with standard morphine solutions. The total amount (free and bound) of residual morphine present in the P_2 fraction, 0.5–1.5 pmol per mg protein, was such that its concentration was less than 10^{-9} M when diluted as in our standard assay. Such concentrations could not affect our results in any important manner. Furthermore, repeated washings of the P_2 fractions did not change our results.

Figure 1 shows the specific binding of dihydromorphine to brain P_2 fractions prepared from normal and morphine-dependent rats. The two sets of data, identical within experimental error, show that both the number and binding affinity of the morphine receptor sites are the same in the control and morphine-dependent animals. Although the data are expressed per mg of protein they are also valid per rat, since the size and protein contents of the brains of the control and morphine-dependent rats were approximately the same.

Specificity of binding was assessed by measuring ^3H -dihydromorphine binding (at 10^{-9} M) at varying concentrations of narcotic analgesics (and other drugs). In all cases, the brain particulate fractions of normal and morphine-dependent animals were indistinguishable with respect to their binding properties. Thus, Fig. 2 presents data showing the competition of morphine for the receptor site and shows the identity of the two types of brain preparations. The narcotic antagonist naloxone also competes in an identical manner for the specific narcotic receptor sites of morphine-dependent and control rats (Fig. 3) as does the anticholinesterase, eserine (Fig. 4).

These experiments provide evidence that morphine dependence (and therefore also tolerance^{4,7}) is not the result of an alteration in the number or of the nature of the specific receptor sites in the brains of rats. Thus, this class of theories cannot account for narcotic drug dependency. In corroboration is the fact that dependent animals, tolerant to large doses of narcotic drugs, still react vigorously to small doses of antagonists such as naloxone or nalorphine^{7,12}. These are believed to bind to the

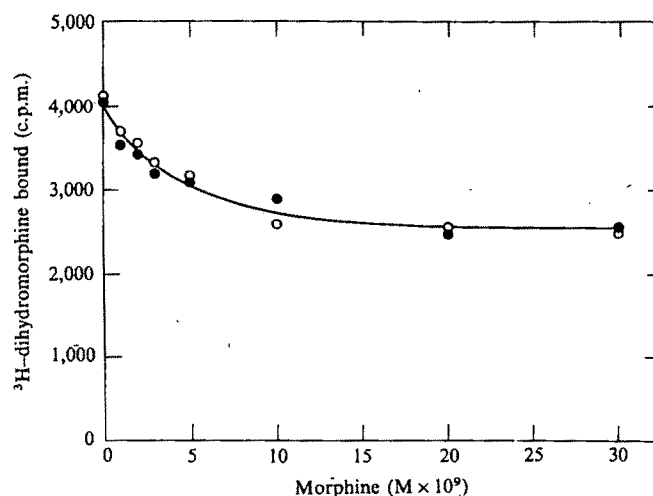


Fig. 2 The displacement of bound ^3H -dihydromorphine from particulate fractions of rat brain homogenates by morphine. Open circles, control; filled circles, morphine-dependent animals. Each flask in this and the subsequent figures contained 0.5 mg of P_2 protein, 9 μmol Tris-HCl, pH 7.4, 1 pmol ^3H -dihydromorphine (31,000 c.p.m.), and the indicated concentration of unlabelled drug in a total of 1 ml of 0.32 M sucrose. The points are single values, and the binding indicated is per flask.

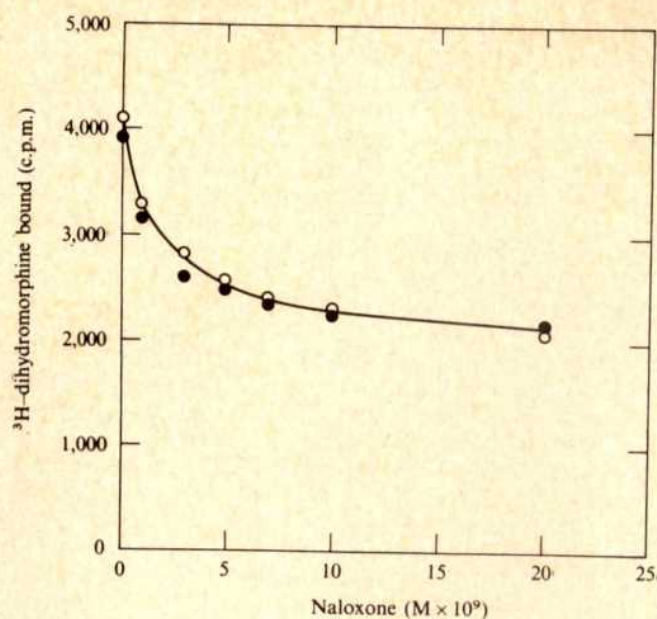


Fig. 3 The displacement of bound ^3H -dihydromorphine from particulate fractions of rat brain homogenates by naloxone. \circ , Control; \bullet , morphine-dependent animals.

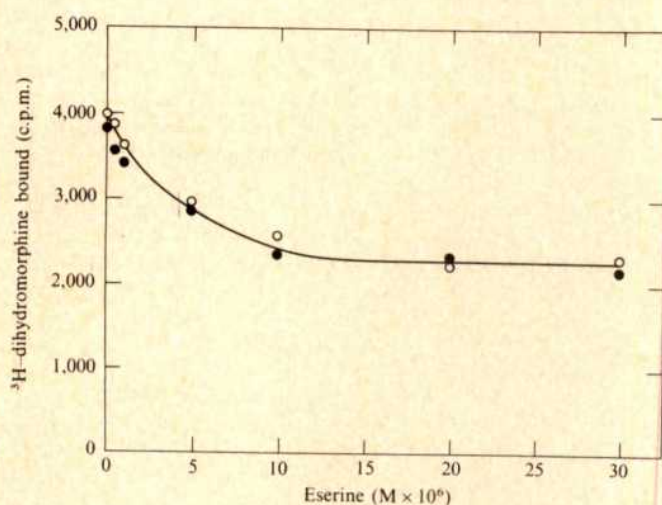


Fig. 4 The displacement of bound ^3H -dihydromorphine from particulate fractions of rat brain homogenates by eserine. \circ , Control; \bullet , morphine-dependent animals.

same receptors as the narcotics themselves on the basis of *in vitro* binding studies⁸⁻¹⁰ and their very close structural similarity to the analgesics. Thus, if tolerance and dependence were simple the result of an increased number (or decreased affinity) of receptor sites much larger doses of antagonists should be required.

A body of evidence indicates that increased breakdown or neutralization of narcotics is also not responsible for tolerance. Thus, both brain and circulating levels of narcotic analgesics are generally similar or identical in normal and tolerant animals after administration of the same dose of a narcotic analgesic^{16,17}.

Apparently, narcotic drugs reach the brain and are bound at receptor sites to the same extent in both normal and tolerant animals. Tolerance and dependency therefore reflect a change in some subsequent process. Conceivably, the efficacy with which receptor binding is translated into the primary physiological response is greatly reduced in tolerant animals. Alternatively, physiological pathways which function in opposition

to the morphine-induced response may be greatly enhanced. This homeostatic mechanism is attractive in that it can easily predict the presence of strong withdrawal syndromes.

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Toxic action of a phalloidin-albumin conjugate on cells with a high protein uptake

PHALLOIDIN (PHD) is a toxic cyclopeptide in *Amanita phalloides*^{1,2}. Rats and mice injected with this toxin die in 2-4 h from hepatic necrosis. Dilatation of the endoplasmic reticulum (ER) with the appearance of very large vacuoles are the first ultrastructural changes in hepatocytes^{3,4}. The first biochemical lesion produced by PHD is unknown². Hepatocytes are the only cells damaged by PHD in mice and rats^{5,6}; Heart fibroblasts⁷ and bovine kidney cells⁵ cultured *in vitro* are not affected by this toxin.

Two possible explanations why PHD damages hepatocytes and not other cells are: (1) PHD enters only hepatocytes; (2) only within these cells does it find the target for its toxic action.

In previous experiments (ref. 8 and E. Bonetti, M. D. and L. F., unpublished) it was found that amanitin-albumin conjugates produce the characteristic changes of amanitin poisoning in cells which are not damaged by the free toxin^{8,9}, but which are very active in protein uptake such as the sinusoidal liver cells of mouse¹⁰ and the kidney proximal tubule cells of rat^{11,12}. This finding demonstrated that these cells are susceptible to amanitin but that the toxin is unable to penetrate them unless it is coupled to a protein and so forced to enter by pinocytosis.

We have conjugated PHD to bovine serum albumin (BSA) and studied the effects of the conjugate (PHD-BSA) on macrophages cultured *in vitro* and also on liver sinusoidal cells and kidney proximal tubule cells of mice. The con-

jugation of PHD with BSA was performed as described for the coupling of amanitin to BSA⁸. The molar ratio of phalloidin to albumin in PHD-BSA was calculated according to Wieland and Buku¹³ and was found to be 2.3.

The effects of PHD and of PHD-BSA on mouse peritoneal macrophages are reported in Table 1. It shows that conjugation with BSA produces a very strong increase in toxicity of PHD for these cells.

TABLE 1 Effect of PHD and PHD-BSA on mouse peritoneal macrophages.

$\mu\text{g ml}^{-1}$ *	PHD		$\mu\text{g ml}^{-1}$ *	PHD-BSA	
	% dead cells			% dead cells	
100	25		400 (12) [†]	100	
50	10		200 (6)	100	
25	0		100 (3)	75	
12.5	0		50 (1.5)	75	

Mouse peritoneal macrophages, obtained as previously described¹⁴, were incubated at 37°C in 0.5 ml of Eagle's basal medium (BME) containing PHD or PHD-BSA. After 15 h 2 ml of BME with 10% foetal bovine serum were added and after further 9 h of incubation the number of dead cells was determined by staining with Trypan blue¹⁵. In the PHD-BSA experiment most of the cells were detached from the glass at the end of the incubation period.

* Concentration during the first 15 h of incubation.

[†] In parentheses the amount of PHD contained in PHD-BSA.

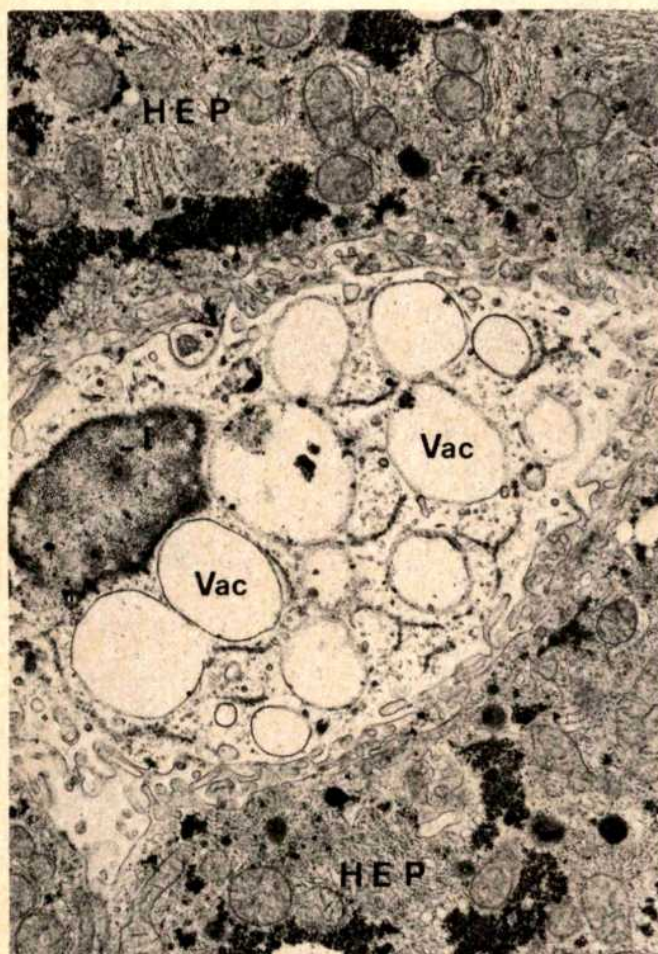


FIG. 1 Mouse killed 8 h after injection of PHD-BSA. Very large vacuoles (Vac) surrounded by a single membrane occupy large part of the cytoplasm of a liver sinusoidal cell. The cytoplasm of hepatocytes (Hep) looks normal. Fixation: 1% osmium tetroxide dissolved in 0.1 M Sorensen buffer, pH 7.2. Embedding: Araldite-Epon mixture according to Mollenhauer¹⁶. Staining: lead citrate. $\times 11,000$.

To study the effects of PHD-BSA on liver sinusoidal cells and kidney proximal tubule cells, Swiss male mice were killed 8 or 24 h after intraperitoneal injection of PHD-BSA dissolved in 0.9% NaCl at a concentration of 800 $\mu\text{g ml}^{-1}$ and given at the non-lethal dose of 80 μg per 10 g body weight ($=2.3 \mu\text{g PHD}$). Specimens of liver and kidneys were observed through the electron microscope.

Eight hours after injection of PHD-BSA, liver sinusoidal cells seemed damaged; the cisternae of rough ER were dilated, forming vesicles of different sizes. In some cells huge vacuoles surrounded by a single membrane occupied a large part of the cytoplasm (Fig. 1). They contained a fibrillar substance of low density, which was scattered in finely divided form. Many sinusoidal cells seemed detached from underlying hepatocytes whose microvilli jutted out directly into the sinusoidal lumen. No morphological alterations were found in nuclei. The hepatocytes seemed unchanged. Twenty-four hours after injection of PHD-BSA liver sinusoidal cells looked normal; they bordered the sinusoidal lumen everywhere.

In kidney PHD-BSA caused changes in the cells of the proximal convoluted tubules. The cells of every other portion of the renal tubular system were unaffected. This agrees with the finding that after injection of albumin in the mouse the only cells of renal tubular system which take up the protein are those of the proximal convoluted tubules¹¹. In these cells a marked increase in smooth ER was observed 8 h after PHD-BSA injection. After 24 h in many cells of the proximal convoluted tubules the cisternae of rough

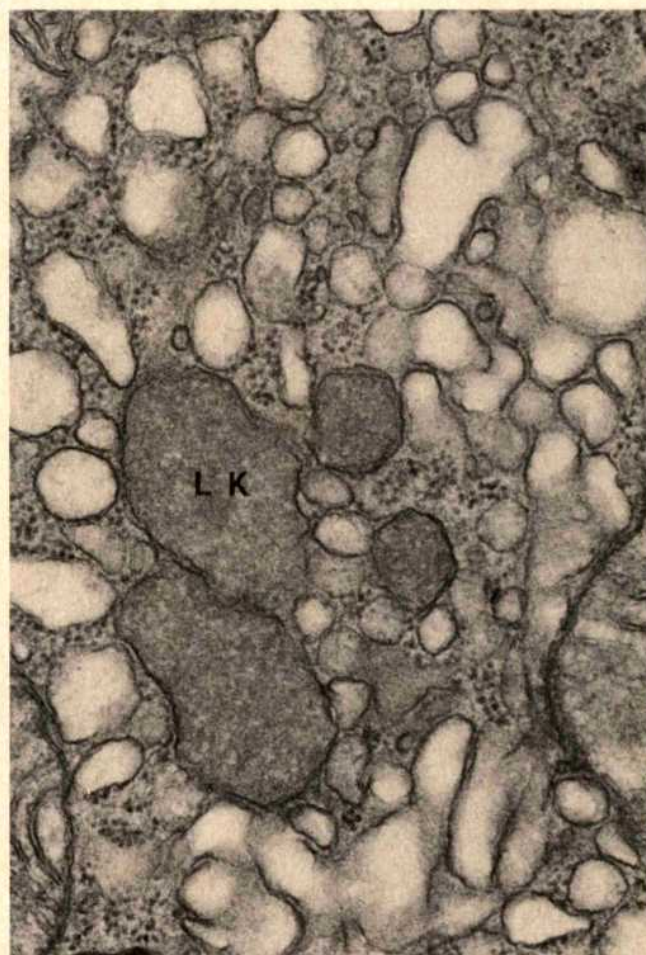


FIG. 2 Mouse killed 24 h after injection of PHD-BSA. Cytoplasm of a proximal tubule cell of kidney. The cisternae of rough ER form vesicles of different sizes. LK indicates *Lipidkörperchen* (see text). Fixation, embedding and staining as in Fig. 1. $\times 45,500$.

ER were dilated, forming vesicles of different sizes (Fig. 2). This lesion is very similar to that observed in hepatocytes 15–30 min after PHD administration^{3,4}. Bodies surrounded by a single membrane, about 0.2 μm in diameter containing moderately dense, homogeneous material, which are a frequent finding in hepatocytes of mouse poisoned with PHD (*Lipidkörperchen*)⁵ were always observed in cells with dilated rough ER (Fig. 2). Some cells instead of the vacuolisation of rough ER showed the increase of smooth ER which is a common finding 8 h after PHD-BSA administration.

Our results show that conjugation with BSA makes PHD toxic for cells with a high protein uptake. The lesions produced by PHD-BSA are very similar to those caused in hepatocytes by free PHD^{3,4}. Since proteins after penetration into cells are rapidly broken down by lysosomal enzymes¹⁷, it seems likely that PHD-BSA exerts its toxic action within the cells after digestion of the protein moiety and release of free PHD from the conjugate. These results indicate also that the absence of lesions in cells different from hepatocytes following PHD administration is due to the inability of these cells to take up the toxin. Therefore the finding that the cells of gastrointestinal mucosa of mice are unaffected after injection of large doses of PHD (20 mg kg⁻¹ i.p.) (our unpublished observations) suggests that the toxin cannot enter these cells and explains why mice are very resistant to the toxin when it is given orally².

These results as well as previous data on amanitin-albumin conjugates (ref. 8 and E. Bonetti, M. D., and L. F., submitted for publication) suggest a possible use of conjugates in biological research. Substances such as hormones and drugs have biological activity only in some cells and sometimes it is difficult to assess whether they enter only the cells in which they are active or whether only within these cells do they find the target of their action. For some of these substances this problem might be solved by conjugating them to a protein and by testing whether in cells with a high protein uptake, the conjugates produce the same effects as are caused by free substances in target cells.

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Sulphur-methylene isosterism in the development of metiamide, a new histamine H₂-receptor antagonist

BURIMAMIDE has been shown to antagonise the effects of histamine on isolated cardiac and uterine muscle *in vitro*, and to antagonise histamine-stimulated acid secretion *in vivo* in animals and in man¹. This pattern of pharmacological effects is not achieved by conventional, tertiary amine, anti-histaminic drugs (of which mepyramine is typical) and has led to the definition of burimamide as an H₂-receptor antagonist¹ and mepyramine as an H₁-receptor antagonist². Although burimamide was pharmacologically sufficiently active to allow for definition of its properties in man³ it seemed to lack the combination of high specific activity with adequate oral bioavailability needed for exploring the therapeutic potential of this new type of drug action. Attempts to produce a more suitable drug were based on the observation that burimamide is a competitive antagonist to histamine and that both compounds possess imidazole rings. We therefore compared the relative species populations of the respective rings and modified the burimamide structure so as to increase the equilibrium concentration of imidazole species considered most likely to be active.

In aqueous solution at physiological pH (7.4) burimamide exists as an equilibrium mixture of mainly three different imidazole species: the two uncharged tautomers A and B, and the cation C (Fig. 1, R = -(CH₂)₄NHCSNHCH₃).

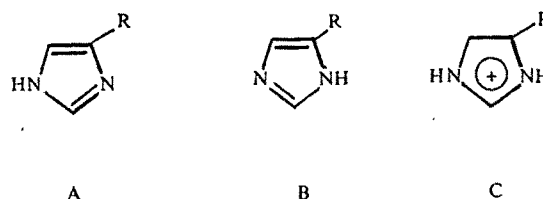



FIG. 1 Three species of imidazole with a side chain R at position 4(5).

Moreover this equilibrium probably involves a water-mediated proton transfer which is not instantaneous, for example protonation of imidazole at pH 7 has a pseudo-first-order rate constant of $1.5 \times 10^3 \text{ s}^{-1}$ (ref. 4). If only one of these forms were active its relative population could determine the amount of drug required for a given effect. The populations of these species are determined by the relative acidities (pK_a) of the two ionisable protons in the cation, but these are not directly measurable. They can be estimated, however, from the electronic influence of the side chain. The substituent R alters the electron densities at the ring nitrogen atoms and affects proton acidity⁵. Its effect is more marked at the nearer nitrogen atom so that if R is an electron releasing group, tautomer B should predominate; if it is an electron withdrawing group, A should predominate. The fraction present as cation C is determined by the ring pK_a and the pH of the solution⁶. The electronic influence of the side chain can be assessed from the measured ring pK_a using the Hammett equation⁷: $pK_{aR} = pK_{aH} + \rho\sigma_m$, that is, the pK_a of the ring substituted by R is reduced relative to imidazole (R = H) in direct proportion to the electron with-

TABLE 1 Apparent pK_a values of substituted imidazole cations at 37° C and their mol fractions (n_c) at pH 7.4

					
	R ₁	R ₂	pK_a	Preferred tautomer	n_c at pH 7.4
Histamine	H	$-\text{CH}_2\text{CH}_2\text{NH}_3^+$	5.90	A	0.03
Thiaborimamide	H	$-\text{CH}_2\text{SCH}_2\text{CH}_2\text{NHCSNHCH}_3$	6.25	A	0.07
Metiamide	CH_3	$-\text{CH}_2\text{SCH}_2\text{CH}_2\text{NHCSNHCH}_3$	6.80	A	0.20
Imidazole	H	$-\text{H}$	6.80		0.20
Burimamide	H	$-(\text{CH}_2)_4\text{NHCSNHCH}_3$	7.25	B	0.40
4(5)-Methylimidazole	H	$-\text{CH}_3$	7.40	B	0.50
Methylburimamide	CH_3	$-(\text{CH}_2)_4\text{NHCSNHCH}_3$	7.80		0.72

pK_a determined potentiometrically at 25° C on 0.005M solutions in 0.1 MKCl by titration against HCl, corrected to 37° C by subtracting 0.0225 units per ° rise for values in the range 7.5 to 7.0 (ref. 11), and 0.02 for the range 6.5 to 6.0 (ref. 12) and rounded off to the nearest 0.05 unit.

drawing effect (σ_m) of R (the reaction constant, ρ , being negative). The pK_a values of relevant compounds are in Table 1.

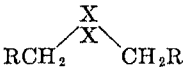
For histamine the ammonium-ethyl side chain ($R = -\text{CH}_2\text{CH}_2\text{NH}_3^+$) lowers the pK_a of the imidazole ring: it withdraws electrons and favours tautomer A. There is only a small proportion of cation C present at pH 7.4 (mol fraction $n_c = 0.03$); the main species is tautomer A, to the extent that approximately 80% of histamine molecules ($n_a = 0.80$) are in this form⁸. For burimamide the ring pK_a is greater than for imidazole, therefore the side chain ($R = -(\text{CH}_2)_4\text{NHCSNHCH}_3$) is electron releasing and tautomer B should be favoured. Electronically the side chain must resemble a methyl group since the pK_a is close to that of 4(5)-methylimidazole. At pH 7.4 the cation is one of the main species ($n_c = 0.40$) and tautomer A should be the least favoured.

Thus, although both histamine and burimamide are mono-substituted imidazoles, the structural similarity is misleading in that the predominant species of the respective imidazole rings are chemically different. If the active form of the antagonist were species A, the form most preferred for histamine, then activity might be enhanced by increasing its relative population. This could be done by converting the antagonist side chain into an electron withdrawing group, for example, by incorporating an additional electronegative atom in the side chain, preferably near to the ring. The disturbance to other molecular properties, such as stereochemistry and lipid-water partition, should however be kept minimal. One way would be to replace a methylene group ($-\text{CH}_2-$) by a thioether linkage ($-\text{S}-$). The data in Table 2 show that these are almost isosteric; they have similar van der Waals radii and give rise to similar bond

angles. The C-S bond is somewhat longer than a C-C bond so that in the thioether the adjacent methylene groups would be farther apart by 0.3 to 0.4 Å; this is quite a modest effect, amounting to an increase in distance between these two groups of about 15%. The thioether linkage may slightly increase conformational flexibility since the energy barrier to rotation of the C-S bond in the thioether linkage is lower than that of the C-C bond in the corresponding hydrocarbon. A sulphur atom is probably more hydrophilic than a methylene group; the octanol-water partition coefficient of diethyl sulphide ($\log P = 1.95$) suggests that the sulphur atom makes no additional contribution to the partition. Comparison with pentane ($\log P = 2.50$) shows that in this simple case replacement of CH_2 by S reduces partition by 0.5 log units, which amount is equivalent to the removal of a methyl or methylene group.

Making this substitution in burimamide at the carbon atom next but one to the ring gave the compound 'thiaborimamide' ($R = -\text{CH}_2\text{SCH}_2\text{CH}_2\text{NHCSNHCH}_3$). The effect on partition of the uncharged molecule is small. $\log P$ (at 37° C between 1-octanol and aqueous buffer at pH 9.0) is reduced from 0.39 (burimamide) to 0.16 (thiaborimamide). The electronic influence of the modified side chain, shown by the ring pK_a , is similar in magnitude but in the opposite direction to that of a methyl group. The main species should be tautomer A although its population may not be as large as it is in histamine because the electronic effect of the side chain is not as marked. A further increase in the population of tautomer A should be obtained by introducing an electron releasing substituent such as methyl in the vacant 4(5)-position of the ring, since electron releasing groups favour the form with the hydrogen atom on the adjacent nitrogen. The methyl group should not interfere with receptor interaction since it has been shown that 4-methylhistamine is an effective H_2 -receptor agonist¹. It might, however, exert an influence on the stereochemistry of the molecule by restricting rotation of the ring in a way analogous to that suggested to occur with 4-methylhistamine⁹. Incorporating methyl into the ring of the antagonist gave N-methyl-N'-[2[(5-methylimidazol-4-yl)methylthio]ethyl]thiourea, which has the name metiamide (name approved by the British Pharmacopoeia Commission and the US Adopted Names Council). The two ring substituents in metiamide are seen to have electronic effects of equal magnitude but of opposite direction. They should combine to favour tautomer A but oppose in their effect on ring pK_a ; indeed, they must exactly cancel since the pK_a s of metiamide and imidazole are identical. This means that at pH 7.4 the main species should be tautomer A, as for histamine, although there would still be a substantial proportion of cation present ($n_c = 0.20$). In comparison with burima-

TABLE 2 Comparison of methylene and thioether linkages

			
	X = CH ₂	X = S	Reference
C—X bond length (Å)	1.54	1.81	13, page 224
CXC bond angle, R = H	109°	105°	13, page 112
van der Waals radius of X (Å)	2.0	1.85	13, page 260
C...C interatomic distances			
between centres (Å)	2.51	2.87	
molar volume increment of X	16.58	10.78	14
C—X rotational barrier,			
R = H (calorie mol ⁻¹)*	3.3	2.13	15, 16
$\log P$ (octanol-H ₂ O), R = CH ₃	2.50	1.95	17, 18

* 1 calorie = 4.186 J

TABLE 3 H₂-Receptor antagonist activities. The dissociation constant (K_B) was calculated from the equation $K_B = B/(x - 1)$, where x is the respective ratio of concentrations of histamine needed to produce half-maximal responses in the presence and absence of different concentrations (B) of antagonist, and $-\log K_B = pA_2$.

	Atrium K_B (95% limits) $\times 10^{-6}$ M	Uterus K_B (95% limits) $\times 10^{-6}$ M
Metiamide	0.92 (0.74 - 1.15)	0.75 (0.40 - 1.36)
Thiaborimamide	3.2 (2.5 - 4.5)	3.2 (2.5 - 4.5)
Burimamide	7.8 (6.4 - 9.6)	6.6 (4.9 - 8.3)
Methylburimamide	8.9 (5.6 - 15)	10.7 (4.5 - 31)

mid, the ratio of tautomers is reversed for metiamide and the proportion of cation is decreased.

The pharmacological consequences of these manipulations are shown in Table 3. The effectiveness of each compound as an H₂-receptor histamine antagonist was compared by estimating the dissociation constant, K_B , (with 95% confidence limits) for the drug-receptor complex. The reliability of these estimates was tested by making separate measurements on two tissues, heart muscle and uterine muscle, which came from different animal species and which respond to histamine in different ways; heart muscle is stimulated and uterine muscle is inhibited. Further, the antagonism was shown to be specific by the failure to antagonise isoprenaline which apparently has identical pharmacological effects to histamine in these tissue¹. The results obtained on the two tissues are in good agreement and, taken together, show that metiamide *in vitro* is three to four times more active than thiaborimamide and eight to nine times more active than burimamide.

Thus it can be seen that modifying the side chain in burimamide by isosteric replacement of $-\text{CH}_2-$ by $-\text{S}-$ favours tautomer A, reduces the ring pK_a , and gives a more active compound (thiaborimamide). Introducing a 4(5)-methyl group to give metiamide increases further the preference for tautomer A but decreases the combined populations of the uncharged tautomers (A and B) through raising the ring pK_a . But although these are opposing effects the net result is that metiamide is more active still. By contrast, the analogous structural modification of burimamide, incorporation of a ring 4(5)-methyl group to give 'methylburimamide' does not increase activity. In this case the two ring substituents have nearly equal electronic effects in the same direction; the methyl group counterbalances the electronic influence of the side chain on tautomerism so that the two tautomers become equally populated, but it raises the ring pK_a to 7.80 so that at pH 7.4 the predominant species is the cation C ($n_c = 0.72$). This illustrates the problem of attempting to manipulate the biological properties of drug molecules through altering the structure. The changes in chemical properties accompanying structural modification often impose their own inherent limitations; a structural change, biologically advantageous with respect to a given chemical property, may affect some other chemical property in a biologically disadvantageous way and one has to discover the optimum balance of opposing influences. Finally, metiamide is not only a more active H₂-receptor antagonist than is burimamide *in vitro*, it is also more active *in vivo*. Metiamide has sufficient oral activity in animals and man as an inhibitor of evoked gastric acid secretion for consideration in therapy¹⁰.

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Similarity in the Sequence of *Escherichia coli* Dihydrofolate Reductase with Other Pyridine Nucleotide-requiring Enzymes

DIHYDROFOLATE reductase (DHFR) is a NADPH-requiring enzyme, and as isolated from a methotrexate-resistant strain of *E. coli* B^{1,2}, has two binding sites for NADPH³. The sequence of DHFR (Fig. 1) has been determined recently in our laboratory by standard methods including CNBr cleavage, enzymatic digestion, and manual Edman degradation with direct determination of the phenylthiohydantoin by gas chromatography and thin-layer chromatography. Novel methods used included free flow electrophoresis instead of ion exchange chromatography for the separation of peptides from tryptic digestion, and cellulose acetate electrophoresis to monitor separations of the peptides produced by CNBr. Details of this work will be reported elsewhere (C. D. B., J. A. Rodkey and J. M. Sondey, manuscript in preparation).

Engel⁴ has pointed out the similarities between the sequence of amino acids around the lysine active in cofactor-binding of glutamate dehydrogenase (GDH) with a second sequence in GDH, which he suggested might be the site of 'regulator'

```
Met-Ile-Ser-Leu-Ile-Ala-Ala-Leu-Ala-Val-
Asp-Arg-Val-Ile-Gly-Met-Glu-Asn-Ala-Met-
Pro-Trp-Asn-Leu-Pro-Ala-Asp-Leu-Ala-Trp-
Phe-Lys-Arg-Asn-Thr-Leu-Lys-Asp-Pro-Val-
Ile-Met-Gly-Arg-His-Thr-Trp-Glu-Ser-Ile-
Gly-Arg-Pro-Leu-Pro-Gly-Ser-Lys-Asn-Ile-
Ile-Leu-Ser-Ser-Gln-Pro-Gly-Thr-Asp-Asp-
Arg-Arg-Val-Thr-Trp-Val-Lys-Asn-Val-Asp-
Glu-Ala-Ile-Ala-Ala-Cys-Gly-Gln-Val-Pro-
Met-Val-Ile-Gly-Gly-Gly-Arg-Val-Tyr-Glu-
Gln-Phe-Leu-Pro-Lys-Ala-Gln-Lys-Leu-Tyr-
Leu-Thr-His-Ile-Asp-Ala-Glu-Val-Asp-Gly-
Asp-Thr-His-Phe-Pro-Asn-Glu-Tyr-Pro-Glu-
Trp-Glu-Ser-Val-Phe-Ser-Glu-Phe-His-Asn-
Ala-Asp-Ala-Gln-Asn-Ser-His-Tyr-Cys-Phe-
Lys-Ile-Leu-Glu-Arg-Arg
```

Fig. 1 Sequence of dihydrofolate reductase of a mutant of *E. coli*.

* Residues known to be active in cofactor binding. The residue position in the whole protein is given above each sequence.

Fig. 2 Comparison of partial sequences of pig glyceraldehyde-3-phosphate dehydrogenase (G3PDH), horse alcohol dehydrogenase (ADH), the area of beef glutamate dehydrogenase suggested to be the regulator sequence (GDH-R), the same enzyme containing the lysine active in cofactor binding at 126 (GDH-A), *E. coli* dihydrofolate reductase (DHFR), and dogfish lactate dehydrogenase (LDH). Homologies with DHFR are shown in boxes.

Current studies of active site labelling, additional sequences of enzymes requiring a pyridine nucleotide as a cofactor and

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In normally innervated adult muscle, membrane sensitivity to acetylcholine (ACh) is localised to the endplate and tendon regions^{1,2}. After surgical denervation, this sensitivity spreads over the entire membrane surface reaching a peak within 5 to 7 d (ref. 3). Two hypotheses have been proposed to explain the restriction of ACh sensitivity in normally innervated muscle. The first suggests that a trophic substance is released from the motoneurone, restricting transmitter sensitivity to the endplate region^{4,5}. The second hypothesis suggests that muscle 'activity' generates a self-feedback loop from the muscle onto the sarcolemma thus limiting ACh sensitivity to the neuromuscular junction^{3,6}. Upon denervation, muscle 'activity' ceases and therefore the feedback loop is broken with the consequent loss of activity-mediated transmitter sensitivity restriction. Drachman and Witzke⁷ showed that direct electrical

stimulation of the denervated rat diaphragm, resulting in contraction, suppressed the spread of ACh supersensitivity. Jones and Vrbová⁶ reported that chronic electrical stimulation of the extensor digitorum of rat, resulting in contractions, reduced supersensitivity to ACh subsequent to denervation.

If muscle 'activity' is responsible for limiting ACh sensitivity to the endplate region, then feedback information from the muscle could emanate either from the contractile event or from membrane events in the absence of excitation-contraction coupling. To determine if mechanical activity, *per se*, is a necessary condition for limiting ACh sensitivity, or whether fluctuations in membrane potential alone constitute a sufficient signal for transmitter sensitivity restriction, we have stimulated denervated muscle above and below the mechanical threshold.

Both extensor digitorum longus (EDL) muscles from albino rats weighing 300 to 400 g were denervated by removal of a 1 to 2 cm section of the deep peroneal nerve between the EDL and the knee⁵. Teflon coated, multistrand wire (32 gauge) was wrapped once around the tendon of origin and insertion and secured by a nylon ligature to nearby connective tissue. The wire was fed through a small diameter polyethylene tube, previously introduced subcutaneously from the thigh to the neck, where it was brought to the surface for connection to a stimulator. One EDL of the first group of animals was electrically stimulated with 5 ms pulses, at a frequency of 15 pulses s⁻¹ and an amplitude of 6.6 ± 0.1 V for 1 h d⁻¹ over a period of 4 d commencing 24 h after denervation. Stimulus intensity was adjusted to a level resulting in maximal contractions as observed with a surgical microscope at $\times 80$ magnification. The other

1×10^{-5} M ACh. After a washout period of 5 min in RR, the muscles were challenged with a solution containing 1×10^{-3} M ACh. Isometric tension was measured with two RCA 5734 mechano-electric transducers and recorded on a Hewlett-Packard paper recorder. Tension measurements were standardised by calculating tension per unit wet muscle weight. The results from paired muscles are shown in Table 1. The data show that sensitivity to ACh from subliminally stimulated muscles is consistently decreased by up to 38% when compared to sham stimulated muscles. The data also indicate that there is no difference between subliminally stimulated and maximally activated muscles.

On this basis we conclude that the feedback information from the muscle onto its sarcolemma is not necessarily mediated by activation of the contractile machinery itself. Muscle membrane potential fluctuations, induced by subliminal electrical stimulation, are therefore sufficient to restrict the spread of denervation-induced transmitter supersensitivity. The reduction of supersensitivity by subliminal stimulation must represent a minimum value as it is quite likely that centrally located fibres did not receive sufficient current to be affected. Therefore, if we were to eliminate the contribution to ACh sensitivity of these centrally located fibres, the reduction in sensitivity would be even greater than we report here. This consideration, no doubt, also contributes to the rather large variability in the response of different muscles owing primarily to the different current densities which depend on muscle geometry. Our results confirm earlier reports^{3,7} that electrical stimulation of denervated muscle is effective in limiting the spread of denervation-

Table 1 ACh mediated isometric tension of paired denervated muscles

Test solution *	Sham †	Against	Subliminal	% Difference	Maximal	Against	Subliminal	% Difference
1×10^{-5} M ACh	$3.1 \pm 0.5 \ddagger$	$n=5$	1.9 ± 0.4	-38§	1.4 ± 0.4	$n=11$	1.4 ± 0.6	0
1×10^{-3} M ACh, first challenge	33.1 ± 7.2	$n=5$	26.6 ± 6.3	-20	11.4 ± 1.5	$n=11$	11.3 ± 1.9	0
1×10^{-3} M ACh, second challenge	12.6 ± 3.3	$n=5$	8.4 ± 4.3	-33	4.0 ± 1.4	$n=11$	3.8 ± 1.0	0

* Acetylcholine was dissolved in rat Ringer solution having the following composition: 160 mM Na⁺, 5 mM K⁺, 2 mM Ca²⁺, 1 mM Mg²⁺, 146 mM Cl⁻, 15 mM HCO₃⁻, 5 mM Tris buffer, 5 mM HPO₄²⁻, and 11 mM glucose at pH 7.1, saturated with 95%–5% O₂–CO₂.

† All muscles were denervated and connected to the stimulator wires in an identical manner. No stimulating current was delivered to the sham muscles. Stimulated muscles received trains of rectangular pulses lasting 5 s. Pulses within the trains had a duration of 5 ms and a frequency of 15 s⁻¹. The amplitude of the pulses delivered to the subliminal muscles was adjusted to a level just below any visually detectable mechanical activity, whereas maximal muscles received stimuli whose amplitude resulted in maximal mechanical activation. *n*=number of muscle pairs.

‡ Values given are means \pm S.E.M. in g tension per g wet muscle weight.

§ Significant at the 0.05 level.

EDL of each animal was stimulated in the same manner except that stimulus intensity was adjusted to just below the mechanical threshold (3.9 ± 0.1 V) as judged from frequent microscopic observations of superficial fibres.

One muscle from a second group of animals was stimulated for 48 h continuously with trains of square pulses lasting 5 s. Pulses within the train had a duration of 5 ms, a frequency of 15 s⁻¹ and a pulse amplitude of 2.9 ± 0.3 V. This amplitude was just below the mechanical threshold (subliminal stimulation). The inter-train interval was 7 s. The other muscle of each animal served as a control. It was denervated and connected to the stimulating wire as described above except that no current was passed to that muscle (sham stimulation). Two hours after the end of the stimulation regime, both muscles from the same animal were excised and mounted in a constant temperature chamber at $37 \pm 1^\circ$ C. The muscles were continuously superfused with rat Ringer (RR) solution saturated with 95% O₂, and 5% CO₂ at a flow rate of 40 ml min⁻¹ (Table 1). The muscles were stretched to a resting tension of 5 g and equilibrated in RR for 5 min before being challenged with RR solution containing

induced supersensitivity. In addition, our data favour the hypothesis that sarcolemmal sensitivity to ACh is regulated by a mechanism located in or near the muscle membrane. This mechanism could be activated by fluctuations in membrane potential without the necessary involvement of excitation-contraction coupling.

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Role of Muscle Activity in Nerve-Muscle Interaction *in vitro*

CLONAL nerve and muscle cells interact *in vitro* to produce a nerve-muscle contact which is associated with an area of increased acetylcholine sensitivity on the muscle^{1,2}. This association is reminiscent of the distribution of acetylcholine sensitivity on innervated skeletal muscle *in vivo*^{3,4}. It is possible to use cultures of nerve and muscle cells to examine the requirements for localisation *in vitro*. A previous report showed that cholinergic transmission is not required². Here I discuss the requirement for muscle electrical and contractile activity in localisation of acetylcholine sensitivity.

To examine the requirement for muscle activity in the events leading to localisation, it was necessary to block the electrical and contractile activity in muscle fibres. The action potential is resistant to 3×10^{-7} M tetrodotoxin⁵ and 5 mM procaine was toxic on prolonged exposure. However, both the action potential and delayed rectification are inactivated by prolonged depolarisation (Y. Kidokoro, in preparation), as is the case in frog skeletal muscle fibres⁶. The resting membrane potential is largely determined by a K^+ permeability, so it is possible to depolarise the cells by increasing the external K^+ concentration. When the external K^+ was increased from 5.3 to 25.3 mM by adding sterile 1 M KCl to growth medium⁷, the membrane potential was depolarised to about -40 mV and remained depolarised up to 2 weeks (-39 ± 5 mV, $N=48$). The resting potential in 5.3 mM K^+ is about -70 mV.

Muscle fibres remained depolarised throughout a long term exposure to 25.3 mM K^+ medium, but it was necessary to show that action potentials were still blocked. Accordingly, muscle fibres grown in medium with 25.3 mM K^+ (high K^+) for 10–14 d, were penetrated with two microelectrodes while still in high K^+ . To elicit even a partial action potential, a fibre had to be polarised to at least -70 mV for 100 or more ms

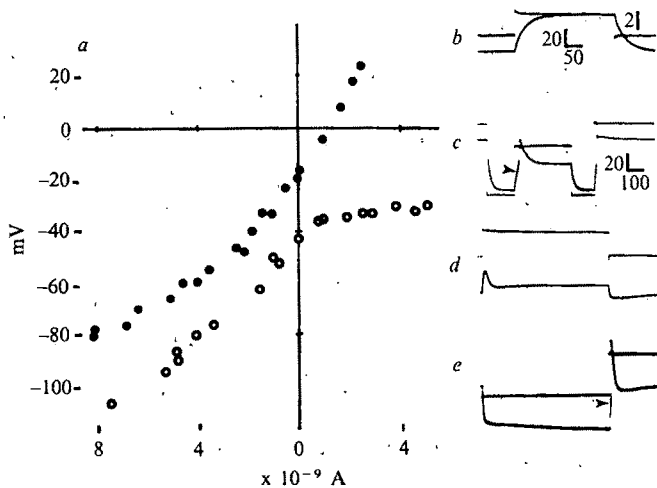


Fig. 1 The steady-state current-voltage relationship (measured 200 ms after the start of the current pulse) for a muscle exposed to high K^+ medium for 12 d and studied in high K^+ , is shown in (a) (solid circles); that for a muscle fibre fused in high K^+ , grown for 14 d, then studied in normal K^+ is shown by hollow circles. Samples traces from the same fibres are shown. (b) Shows the absence of delayed rectification in high K^+ , while (d) shows its presence in the fibre formed and grown in high K^+ . (c) Shows that only a partial action potential can be evoked from this fibre in high K^+ (a hint of the potential can be seen at the end of the hyperpolarizing pulse, but it is more clearly shown by the superimposed depolarization). (e) Shows an overshooting action potential in the fibre fused in high K^+ , then studied in normal K^+ . Note that the inflections in the rising phase of the action potentials occur at -50 to -60 mV (arrows). Calibrations are shown in the figure—the upper trace shows injected current ($\times 10^{-9}$ A) and zero membrane potential, the lower shows membrane potential (mV), time is in ms. The calibrations are the same for (c), (d) and (e).

(Fig. 1c). The steady state current-voltage relationship in high K^+ showed anomalous rectification; rather than delayed rectification (Fig. 1a). No permanent changes had been made in membrane properties, however, since when fibres exposed to high K^+ for 2 weeks were washed and studied in normal K^+ medium, they immediately (that is, the first cell penetrated) could generate overshooting action potentials on anode break stimulation, and showed delayed rectification. Furthermore, myoblasts grew and fused normally in high K^+ . Action potentials and delayed rectification were inactivated in high K^+ in these fibres, as just described for fused fibres chronically exposed to high K^+ . Again, if such fibres (fused in high K^+) were washed and studied in normal K^+ , they also could generate overshooting action potentials (Fig. 1e) and showed delayed rectification (Fig. 1a and d).

Table 1 Average Enhancement of Sensitivity at Nerve-Muscle Contacts

No. of muscle fibres	Ratio of peak localised sensitivity to background (Average)	sensitivity (Range)	No. of sites tested per cell (Average)	(Range)	Success, 0.7
11	18	6–44	23	8–63	0.7

L6 myoblasts⁸ and N18 neuroblastoma cells⁹ were cultured as described previously². The distribution of acetylcholine sensitivity was determined by penetrating a muscle fibre with a 3 M KCl-filled micropipette (70–150 M Ω), then applying acetylcholine iontophoretically from an extracellular pipette filled with 2.7 M acetylcholine chloride (100–300 M Ω)². The acetylcholine sensitivity of the membrane is defined² as the membrane depolarisation (in mV) divided by the amount of charge (in nC) passed through the acetylcholine pipette to produce the response (mV nC⁻¹). The results are pooled for fibres which had the 25.3 mM K^+ medium added before or after myoblast fusion, since localisation occurred in either case. The number of sites tested per cell gives the number of different areas of membrane that were tested with applied acetylcholine. 'Success' is the ratio of the number of muscle fibres that showed localisation to the number of nerve-muscle pairs that were chosen for study on the basis of their appearance in the phase microscope. The site of contact had to be clearly visible and situated so that the acetylcholine sensitivity could be determined all around it, and it had to be possible to trace the nerve process back to a nerve cell body.)

High K^+ medium reversibly stopped the twitching of spontaneously active muscle fibres. Fibres in high K^+ did not contract in response to intracellular stimulation or iontophoretically applied acetylcholine. These observations are consistent with studies on frog muscle fibres, which first contract when exposed to an increased K^+ concentration, then relax and no longer respond to electrical stimulation¹⁰.

To determine whether muscle activity was required in the events leading to localisation of acetylcholine sensitivity *in vitro*, nerve and muscle cells were cultured together in high K^+ medium for 8–12 d. In some studies the high K^+ was added to cultures of myoblasts before they had fused. In all studies high K^+ medium was added before the nerve cells and all electrophysiology was done in high K^+ medium, so that the block of activity was in effect throughout the experiment. Many examples of localisation of acetylcholine sensitivity were found, as seen in Fig. 2 and Table 1. In each case the peak of sensitivity was found at a nerve-muscle contact, and there was a clear gradient of sensitivity across the muscle surface up to the peak (see ref. 2 for further discussion).

I concluded from these results that muscle action potentials and contractile activity are not required for the events leading to the association of a nerve-muscle contact with an area of increased acetylcholine sensitivity on the surface of the muscle in this clonal system. Muscle fibres in primary cultures of chick embryo tissue can generate areas of increased acetylcholine sensitivity¹¹, or receptor density¹² in the absence of nerve cells. It is not known whether the localised sensitivity seen at a point of nerve-muscle contact in clonal cell culture preceded the contact or was induced by the intercellular contact.

The results obtained when high K^+ medium was added to myoblasts before fusion show that, in either case, muscle activity was not required for its production.

Recent studies have shown that electrical stimulation of denervated muscle *in vivo*^{13,14} can reduce the extrajunctional sensitivity, and stimulated muscle fibres *in vitro* are less sensitive than tetrodotoxin-treated fibres¹⁵. These studies strongly suggest that muscle activity influences the extrajunctional sensitivity on muscle fibres. However, results which are difficult to reconcile with the conclusion that muscle activity strictly determines the extrajunctional sensitivity have been reported¹⁶⁻¹⁸, and the relationship between muscle activity and sensitivity is not clear at present. Specifically, although activity reduced the extrajunctional sensitivity of denervated muscle, no effect on junctional sensitivity was noted¹³. It is possible that junctional and extrajunctional sensitivities are controlled by different processes and might be differently affected by muscle activity. It is not known whether the localisation seen in this clonal cell culture involves a decrease in the background sensitivity on the muscle cell (further discussion in ref. 2). Thus, there is no contradiction between the present results and other studies¹³⁻¹⁵.

My results demonstrate that muscle activity is not required for the localisation of acetylcholine sensitivity on these fibres *in vitro*, but do not indicate whether muscle activity has other effects on sensitivity (perhaps on the extrajunctional sensitivity, for example, refs 13-15).

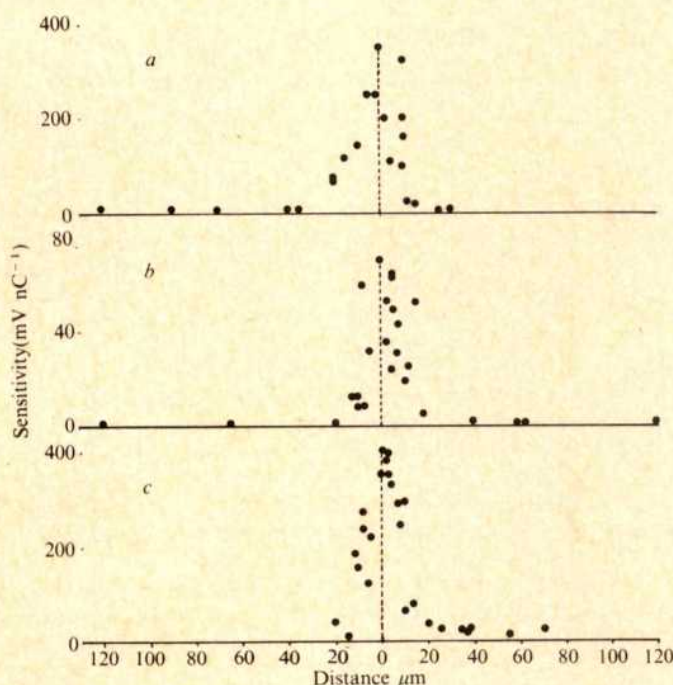


Fig. 2 Examples of localization of acetylcholine sensitivity. The ordinate shows the acetylcholine sensitivity (in $mV nC^{-1}$) detected at various points on the muscle surface, while the abscissa shows the distance in μm between the indicated site and the site with the maximal sensitivity. In each case, the peak of sensitivity was at a nerve-muscle contact. In (a) and (b), the 25.3 mM K^+ solution had been added to cultures of fused muscle fibres; in (c) the 25.3 mM K^+ solution had been added to a culture of myoblasts before fusion.

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Spine Loss and Regrowth in Hippocampus following Deafferentation

SEVERAL studies have reported that there is a loss of dendritic spines after partial deafferentation. It is known that terminals lost to a structure after injury to one of its afferents are replaced in part or whole by sprouting of undamaged inputs¹⁻³. This raises questions as to the nature of the accompanying post-synaptic changes but no attempts have been made to follow the loss of spines over time⁴⁻⁶. Specifically, there are no data on whether spines are replaced when sprouting afferents invade deafferented dendritic zones. This is a critical question if the nature of the morphological reorganisation which follows lesions in the brain is to be understood.

We therefore studied the effects of lesions of the entorhinal cortex on the spine population of neurones in the dentate gyrus. The entorhinal cortex generates the temporo-ammonic tract⁷⁻¹⁰, which contributes more than half of the synapses in the outer molecular layer of the dentate gyrus (D. A. Matthews, C. W. C., and G. L., unpublished). Following its removal, axons of the commissural system (which occupies the inner molecular layer) and the crossed temporo-ammonic tract (which terminates on the apical dendrites of CA1 pyramids) invade the outer molecular layer and form functional terminals¹¹⁻¹⁴. The scattered AChE terminals, probably originating in the septum and located in the outer molecular layer, proliferate after entorhinal lesions¹⁵⁻¹⁶. Thus, considerable replacement of the temporo-ammonic tract terminals occurs in the outer molecular layer after entorhinal lesions. Here we report changes which occur on dendritic spines of cells in the molecular layer while this axonal growth takes place.

Large electrolytic lesions were placed in the entorhinal cortex of adult rats of both sexes. The animals (E) were sacrificed at 5 ($n=4$), 20 ($n=4$), 60 ($n=4$) and 100 ($n=4$) d. An equal number of animals of the same age and approximately of the same weight served as controls (C). The rats were anaesthetised with ether. Tissue was excised from the rostral hippocampus and immediately immersed in rapid Golgi fixative of osmium tetroxide and potassium dichromate for 6 d. After fixation, the blocks were placed in 0.75% silver nitrate for 24 h. They were then 'shelled' in paraffin and cut on a sliding microtome at 75 μm . Following two washes each with absolute ethanol, methyl salicylate and xylene, they were mounted under Per-

mount. The rapid Golgi method silhouettes the soma-dendritic complex in detail including the dendritic spines¹⁷.

Spines were counted in the outer and inner molecular layers of the dorsal leaf of the dentate gyrus ipsilateral to the entorhinal lesions. Counts were performed on 45 μ m segments (stations) on fifteen granule cells per animal (Fig. 1). All measurements in the outer molecular layer were taken from dendritic branches located within 100 μ m of the hippocampal fissure and those for the inner molecular layer were made within 100 μ m of the granule cell layer. As far as possible the same medial-lateral segment of dentate gyrus was examined in each rat.

Spine density was essentially the same in both layers in normal rats (Table 1). Five days after the entorhinal lesion there was a 30% reduction in the number of spines of the outer molecular layer (Fig. 2) but no changes were found in the inner molecular layer. By 20 d after the lesion, spine density had returned to 80% of normal and was back to control levels 60 d after the lesion (Table 1).

Table 1 No. of Dendritic Spines per μ m of Dendrite

Station	Controls (C)	Experimental (E)	% difference*	P†
5 d after lesion				
A	0.64 ± 0.12 ‡	0.60 ± 0.09	-4.7	—
B	0.61 ± 0.10	0.43 ± 0.10	-29.5	<0.005
20 d after lesion				
A	0.60 ± 0.10	0.63 ± 0.10	+3.3	—
B	0.60 ± 0.11	0.48 ± 0.11	-20.0	<0.005
60 d after lesion				
A	0.62 ± 0.12	0.60 ± 0.09	-3.2	—
B	0.60 ± 0.09	0.60 ± 0.11	0.0	—
100 d after lesion				
A	0.63 ± 0.10	0.64 ± 0.11	+1.6	—
B	0.61 ± 0.11	0.60 ± 0.10	-1.6	—

* $100 (E \text{ mean} - C \text{ mean}) / C \text{ mean}$.

† Student's *t* test.

‡ Standard deviation.

It is interesting to compare the time course reported above with that reported for the invasion of axonal sprouts into partially denervated dendritic territory. Raisman and Field reported that the terminal population of dendritic spines in the

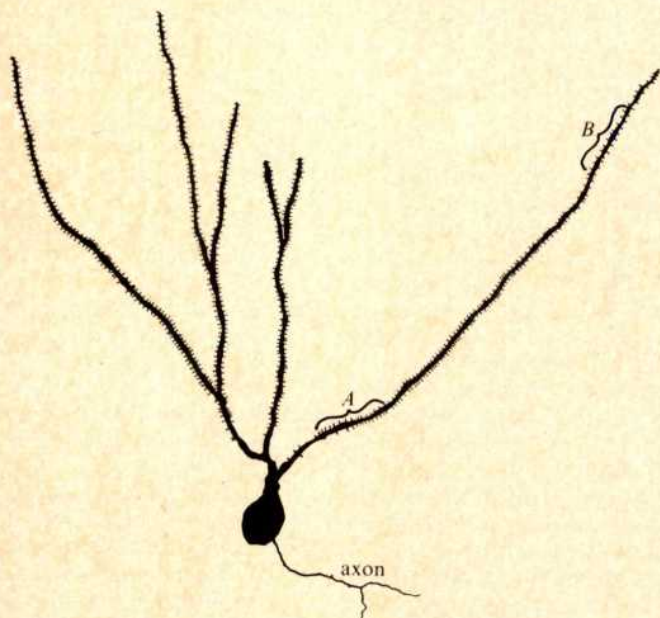


Fig. 1 Counting stations along the dendrites of the hippocampal granule cell. Stations A and B are located in the inner and outer molecular layers of the dentate gyrus respectively.

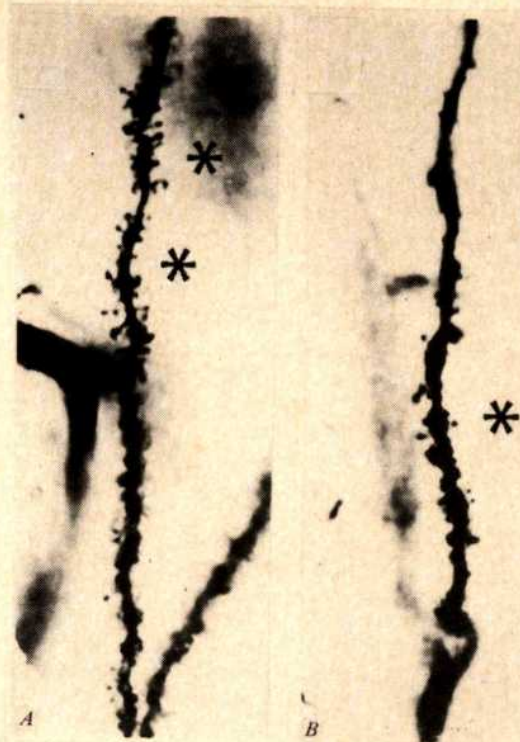


Fig. 2 A, Density of spines in the outer molecular layer of a normal adult rat; B, loss of spines as a result of an electrolytic lesion in the entorhinal cortex 5 d after the operation. * Portions of dendrite in focus.

septal nuclei was reduced to one-half of normal values 7 d after section of their hippocampal projections but returned to normal values by 30 d after the lesion¹⁸. The new terminals are thought to arise in part from sprouting of medial forebrain bundle axons. Neurophysiological studies have shown that the crossed temporo-ammonic¹⁴ and commissural (J. West, S. Deadwyler, C. W. C., and G. L., unpublished) systems establish functional synapses in the outer molecular layer 10 to 14 d after a lesion to the entorhinal cortex. Finally, histochemical experiments indicate that the process of axon sprouting is begun 5 to 7 d after lesions¹⁹. Taken together, these findings indicate that axon sprouting starts within 7 d of a lesion, establishes some functional synaptic contacts by 14 d after the lesion, and is essentially completed within 30 d of the lesion.

Although our data do not provide a precise picture of the time course for spine regrowth it is evident that the process is occurring during the period in which axonal sprouting takes place. This leads to the suggestion that the two processes, namely spine regrowth and axonal sprouting, might be related. Spine formation during development is often hypothesized to be dependent upon the arrival and normal function of axon terminals. We suggest that a similar mechanism occurs in the adult. A reduction of afferents results in loss of spines. Replacement of afferents by sprouting generates a new spine population. A special case of this is data reported by Valverde²⁰ from a study in which he raised mice in complete darkness from birth to 20 d and then placed them under normal lighting conditions for periods of up to 30 d. He claims 'recovery' of some spines on apical dendrites in the visual cortex of these animals—and suggests that the development of this group of spines results from the arrival of normal visual inputs. The possibility exists that there may be little or no correspondence between the two studies.

Our hypothesis, stated above, might also explain why several workers have observed a permanent reduction in spine densities following lesions^{5,6}. There is evidence that post-lesion sprouting is not a universal effect^{21,22}. If some brain regions are not re-

innervated by sprouting after the removal of afferents, then spine loss should be permanent.

Finally, these experiments provide clues as to factors which regulate spine density. The dendritic zones deafferented by the entorhinal lesions are re-innervated by three and possibly more different axonal systems. If spine density were solely dependent on the growth potential of presynaptic elements it would hardly be expected that these diverse inputs would generate the same number of spines as the single afferent they replace. Yet our data indicate that spine densities return to values essentially identical to pre-lesion conditions. A more parsimonious hypothesis is that granule cells in the hippocampus are capable of producing a certain number of spines. Given that an adequate number of terminals is present, these cells will generate this quantity of spines independent of the specific type of afferents.

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Growth Hormone Releasing Factor of Microbial Origin

CHOLERA enterotoxin mediates specific biochemical events in both intestinal and non-intestinal tissues by stimulating adenylyl cyclase and cyclic AMP^{1,2}. Other evidence points to a role for cyclic AMP in regulating the secretion of several hormones by their respective endocrine glands³. These findings have led us to investigate whether cholera enterotoxin affects the secretory function of pituitary cells.

Cholera enterotoxin, an extracellular protein of molecular weight 84,000, was purified from culture filtrates of *Vibrio cholerae*, Inaba 569B, as described earlier⁴, adding Sephadex-G-150 chromatography as a final step. Absence of residual somatic antigen was established by the failure of detoxified enterotoxin preparations to elevate vibriocidal antibody; and absence of choleraenoid, a degradation product of toxin⁵, was shown by acrylamide gel electrophoresis.

Monolayer cultures of enzymatically dispersed cells from whole pituitaries of Charles River CD male rats were prepared essentially according to Vale *et al.*⁶ and Grant *et al.*⁷. The growth medium was Eagle's minimal essential medium containing 10% foetal calf serum plus standard amounts of glutamine and antibiotics, and the test medium was Earle's balanced salt solution plus antibiotics. After incubation of cell monolayers with and without enterotoxin for 3 h at 37° C, replicate culture fluids were analysed for growth hormone, prolactin, luteinising hormone and thyrotropin by specific double antibody radioimmunoassay. Results were evaluated by analysis of variance, using Dunnett's procedure for multiple comparisons.

Treatment of cell monolayers with concentrations of enterotoxin from 6×10^{-8} M to 6×10^{-9} M significantly increased the basal secretions of all four hormones (Table 1). Enterotoxin concentrations from 6×10^{-10} M to 1.2×10^{-13} M, however, preferentially raised the secretion of growth hormone. In each

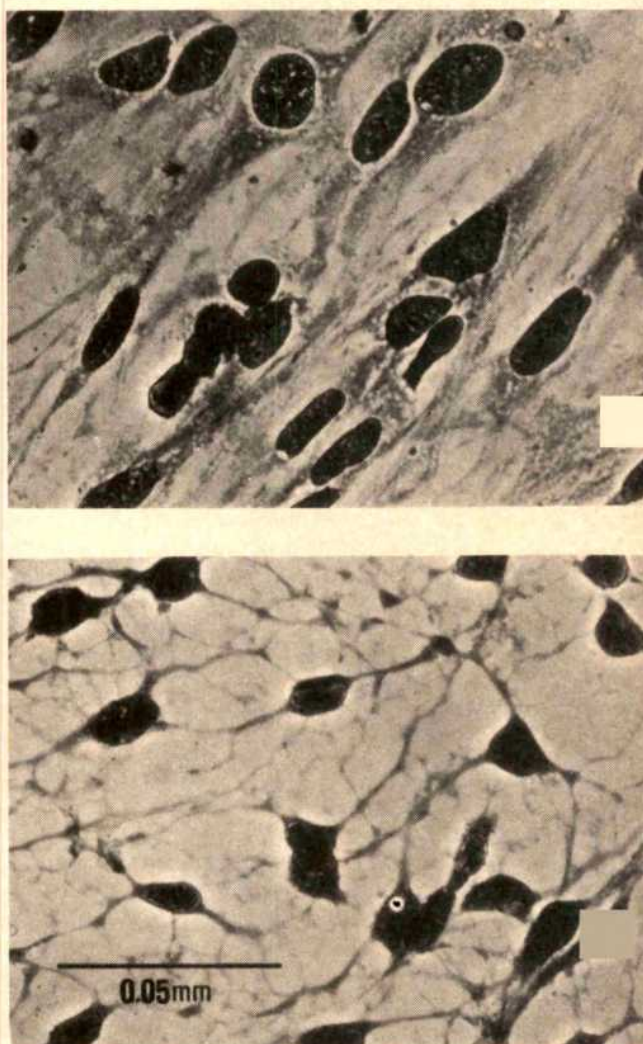


Fig. 1 Monolayer cultures of rat pituitary cells incubated for 3 h at 37° C with test medium (top) or test medium containing 1.2×10^{-11} M of cholera enterotoxin (bottom). Magnification $\times 320$, phase contrast, stained with May-Greewald and Giemsa.)

Table 1 Effect of Concentration of Cholera Enterotoxin on Secretion of Growth Hormone, Prolactin, Luteinising Hormone and Thyroid-stimulating Hormone by Cultured Rat Pituitary Cells

Exp. No.	Cholera enterotoxin concentration (M)	N	Growth hormone	Hormone released (ng ml ⁻¹ , mean \pm s.e.m.)		Thyroid-stimulating hormone
				Prolactin	Luteinising hormone	
1	None	3	436 \pm 20	121 \pm 3	118 \pm 11	505 \pm 89
	6.0 $\times 10^{-8}$	2	543 \pm 44†	173 \pm 6*	270 \pm 28*	1,038 \pm 53*
2	None	3	153 \pm 9	123 \pm 8	40 \pm 4	52 \pm 17
	6.0 $\times 10^{-9}$	2	510 \pm 26*	204 \pm 15*	256 \pm 35*	179 \pm 37
	6.0 $\times 10^{-10}$	2	484 \pm 18*	172 \pm 2	49 \pm 6	195 \pm 76
3	None	3	277 \pm 5	174 \pm 7	107 \pm 5	205 \pm 49
	2.4 $\times 10^{-10}$	2	464 \pm 30*	231 \pm 11*	122 \pm 5	212 \pm 9
	1.2 $\times 10^{-11}$	2	372 \pm 23*	195 \pm 4	88 \pm 6	72 \pm 26
4	None	3	288 \pm 10	133 \pm 11	35 \pm 3	NA
	6.0 $\times 10^{-12}$	2	526 \pm 22*	194 \pm 20	67 \pm 4*	NA
	1.2 $\times 10^{-12}$	2	371 \pm 9†	124 \pm 8	67 \pm 2*	NA
5	None	2	93 \pm 8	88 \pm 8	NA	319 \pm 40
	6.0 $\times 10^{-13}$	2	225 \pm 18*	87 \pm 6	NA	178 \pm 81
	1.2 $\times 10^{-13}$	2	174 \pm 16†	81 \pm 5	NA	241 \pm 58
6	None	3	84 \pm 4	97 \pm 7	15 \pm 1	NA
	6.0 $\times 10^{-11}$	3	397 \pm 54*	131 \pm 5*	17 \pm 1	NA
	6.0 $\times 10^{-12}$	3	258 \pm 26†	117 \pm 5	14 \pm 2	NA
	6.0 $\times 10^{-13}$	3	176 \pm 7	109 \pm 3	12 \pm 2	NA

† P Compared with control value <0.05.

* P compared with control value <0.01.

NA, not assayed; N, number of cell culture dishes, each of which was assayed in duplicate.

experiment, the growth hormone response was dose-related. Neither cholera toxoid⁸ at a concentration of 50 μ g ml⁻¹ nor enterotoxin preincubated with specific antitoxin could increase hormone secretion. In addition, cholera toxin, a competitive inhibitor of enterotoxin⁹, and somatostatin, the growth hormone release inhibitory factor¹⁰, each at a concentration of 2.2 $\times 10^{-8}$ M, depressed the release of growth hormone by 1.2 $\times 10^{-11}$ M enterotoxin.

After treatment with enterotoxin, cells remained viable on the basis both of their ability to exclude trypan blue dye and their ability to be subcultured. Further, enterotoxin treatment produced a striking change in morphology: the cells developed elongated cytoplasmic processes which became noticeable within 1 h of exposure to enterotoxin and which, in some cases, seemed to form webs of interconnecting cells (Fig. 1). Replacement of test medium with growth medium alone reversed the morphology to that of the control cultures. Reintroduction of test medium without enterotoxin, however, led to the same morphological transformation which occurred during treatment with enterotoxin, suggesting that the toxin had been irreversibly bound to the cell membrane. In contrast to enterotoxin-treated cultures, monolayers treated with luteinising hormone releasing hormone (LRH) did not exhibit the transformation.

To determine whether cyclic AMP mediated the secretion of growth hormone by the morphologically transformed cells, control cells and cells treated with 1.2 $\times 10^{-11}$ M enterotoxin were collected by trypsinisation and analysed for intracellular cyclic AMP by the method of Gilman¹¹. Enterotoxin-treated cells, secreting four times more growth hormone than control cells, contained approximately twice the intracellular cyclic AMP found in control cells. Cultures more homogeneous with respect to growth hormone-producing cells would probably have an even greater influence of enterotoxin on cyclic AMP content.

In considering the mechanism governing the preferred release of growth hormone at low concentrations of enterotoxin, we compared the kinetics of this release with the kinetics of a well-defined hypothalamic releasing hormone, LRH. The time course of enterotoxin-induced secretion of growth hormone resembled that of LRH-induced secretion of luteinising hormone: significant release of each hormone failed to appear

before 1 h, but for 2 h afterwards both secretions increased linearly with time. This similarity suggests that the sequence of events leading to secretion may be the same.

These findings provide the basis of a sensitive new assay for cholera enterotoxin. The changed morphology of the pituitary cells may in itself offer a rapid and simple means of detection. Moreover, since cholera enterotoxin comprises a large protein marker with specificity for growth hormone-secreting cells, it should facilitate isolation and characterisation of the particular receptors involved.

Taken with the evidence that cholera enterotoxin elevates steroid production in cultured mouse adrenal cells¹², our findings pose the question of whether the toxin may alter host hormone secretion during the course of the disease. Although systemic penetration by enterotoxin has not been demonstrated in clinical or experimental cholera², a peptide fragment of the molecule, small enough to pass through the intestinal mucosa, may retain the minimum structural requirements for stimulating pituitary receptors.

We thank the National Institute of Arthritis and Metabolic Diseases Rat Pituitary Hormone Distribution Program for radioimmunoassay reagents; anti-rat growth hormone was supplied by Dr R. E. Grindland of the Ames Research Center.

Note added in proof. We have found recently that enterotoxin from two strains of *E. coli* also stimulates growth with an accompanying morphological transformation.

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Histochemical study of an inhibitor of fibrinolysis in the human arterial wall

THE formation of fibrin is a fundamental biological repair mechanism, but the fibrin has ultimately to be removed by fibrinolysis. This is accomplished by the proteolytic enzyme plasmin, which is converted from plasminogen, an inactive precursor in blood, by an activator. Astrup and Permin¹ have demonstrated that the fibrinolytic activity of tissues is due to such an activator of plasminogen.

To localise fibrinolytic activity in different tissues, Todd² developed a histochemical method in which frozen tissue sections are covered by a thin layer of fibrin rich in plasminogen. During incubation at 37° C, structures containing the plasminogen activator caused lysis which appeared as clear zones in the subsequently stained fibrin. By means of this fibrin slide technique, several investigators^{3,4} found the plasminogen activator in human tissues to be concentrated predominantly in endothelial cells of capillaries and veins.

The endothelial cells of the lumen of human arteries usually showed no activity, however, in contrast to the vasa vasorum of their adventitia. This remarkable lack of activity in endothelial cells of human arterial intima prompted us to carry out a renewed investigation of human muscular and elastic

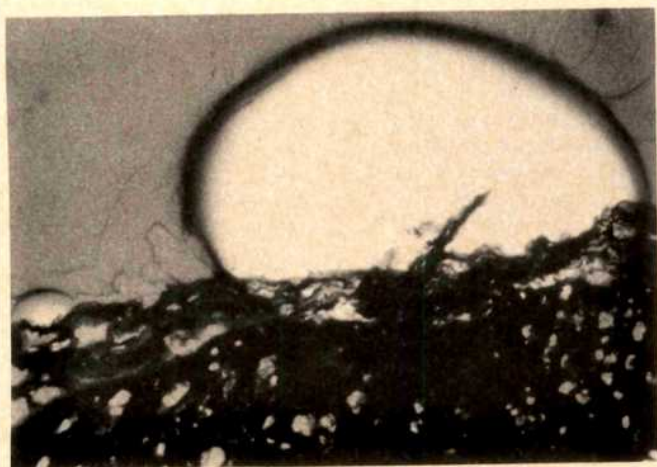


FIG. 1 Lysis produced by the vasa vasorum in the adventitia of the hepatic artery. Left, an initially small zone of lysis; right, an extended zone with flattening towards the media. Harris' alum haematoxylin ($\times 50$).

arteries, as well as veins, by means of the fibrin slide technique. For discrimination between plasminogen activator activity and non-specific protease activity, we prepared slides with bovine fibrinogen with and without plasminogen⁵.

We confirmed the original finding that fibrinolytic activity due to an activator of plasminogen is related to endothelial cells of most veins and capillaries, but not to those of arterial intima. During this investigation, however, our attention was drawn to an outstanding recurrent phenomenon: the initially

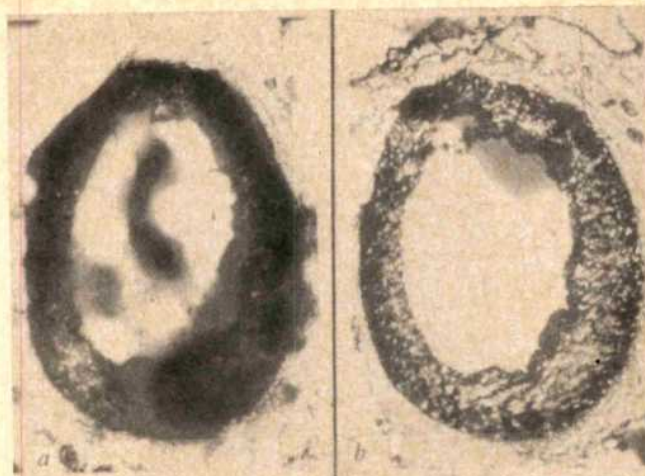


FIG. 2 Cross sections of the splenic artery unheated (a) and heated (b) assayed on plasminogen-free fibrin with the fibrin slide sandwich technique using plasmin as active compounds. After incubation for 140 min, a dark-stained fibrin strand remains on top of the media of the unheated section, while on the heated section all fibrin is lysed. Harris' alum haematoxylin ($\times 5$).

small round areas of lysis due to plasminogen activator in the vasa vasorum of the arterial adventitia flatten towards the site of the media, while they extend during prolonged incubation. Consequently, their centres shift to lateral (Fig. 1). This feature was more pronounced in human muscular than in elastic arteries, while the distortion of lytic zones was hardly noticeable around large human veins. A possible explanation for this could be that medial tissue contains an inhibitor of fibrinolysis which may diffuse into the fibrin layer above it, thus preventing lysis at those sites.

Evidence for this hypothesis was given by demonstrating the inhibition of fibrinolytically active compounds placed on top of the medial site of human arteries. This was achieved by modifying the fibrin slide technique as follows: frozen sections of the tissue to be examined were placed on a microscope slide, covered by fibrin, and left for at least 2 h in a moist refrigerator, to allow diffusion of inhibiting components from the sections into the fibrin layer. Fibrinolytically active frozen sections were then placed on top of the fibrin film after which the preparations were incubated for various lengths of time at 37° C, fixed, and stained. We called this technique the 'fibrin slide sandwich technique'⁶. When used on fibrin films rich in plasminogen, the fibrinolytically active layer of the 'sandwich' consisted of a frozen section of human lung known to be rich in plasminogen activator, or a section of a frozen urokinase solution (human urokinase, Leo Pharmaceuticals, Copenhagen, dissolved to 7 Ploug units ml⁻¹ in saline barbitol buffer with 15% gelatin). Human plasmin⁷ (Michigan Department of Public Health, Michigan, diluted to 1.5 U ml⁻¹ in saline with 15% gelatin) was used on plasminogen-free fibrin.

By means of the sandwich technique using both activator and plasmin as the active layer, a clear inhibition of fibrinolysis was demonstrated in the medial area of fresh human arteries but not in the adventitial region. This inhibitory effect was indicated by a dark-stained fibrin strand remaining on top of the media while the active top layer had lysed most of the fibrin which it contacted. Usually some fibrin is observed remaining in the lumen at the moment when the fibrin adjacent to the adventitia is already lysed (Fig. 2a). Inhibition of both activator and plasmin by human elastic arteries was not as effective as by muscular ones; the walls of large veins, for example the superior vena cava, showed very little inhibition.

During further attempts to confirm our evidence, it was discovered that heating tissue sections in a dry oven at

100° C for 12 h before covering with fibrin abolished the inhibition effect. The absence of a dark-stained fibrin strand on top of the media of a heated section (Fig. 2b) shows the loss of inhibition in contrast to its presence in fresh sections (Fig. 2a).

Our results thus indicate the presence of an inhibitor of fibrinolysis in the human arterial wall which is able to diffuse into fibrin. This provides a natural explanation for the distortion of lytic zones to the medial site, and possibly for the absence of fibrinolytic activity in the arterial intima as seen with the fibrin slide technique. The physiological significance of this inhibitor in human arteries remains to be discovered, but its presence should be kept in mind when considering the role of fibrinolysis in vascular disease.

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Temperature-sensitive Mammalian Cell Line Blocked in Mitosis

SEVERAL temperature-sensitive mammalian cell mutants in culture have been isolated¹⁻¹⁰, including three defective in protein syntheses⁶, cytokinesis⁵ and the processing of ribosomal RNA¹⁰, respectively. I have now isolated a hamster cell mutant in which mitosis is blocked in metaphase.

Hamster cells HM-1 from Kam Laboratories were grown in Dulbecco's modified Eagle's minimal essential medium (MEM) supplemented with 10% calf serum (Grand Island Biological

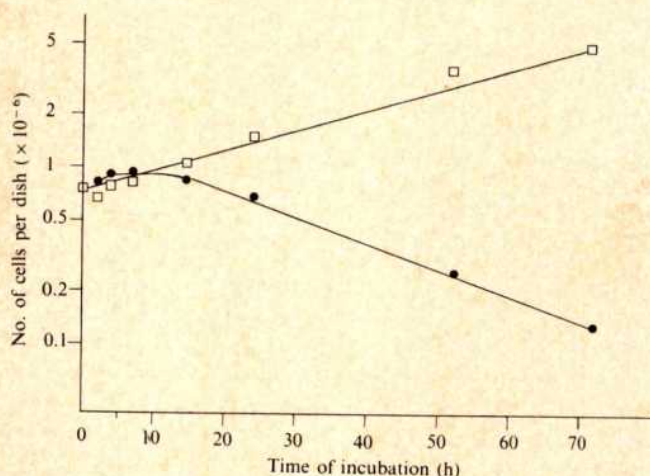


Fig. 1 Growth of ts-546 at 33° and 39° C. Falcon 60-mm culture dishes were inoculated with cells and incubated at 33° C for 3 d. Half the dishes were transferred to 39° C. The medium in two dishes was removed, trypsin added and cell number counted at the time of transfer (time 0) and at intervals thereafter. □, Cells at 33° C; ●, at 39° C.

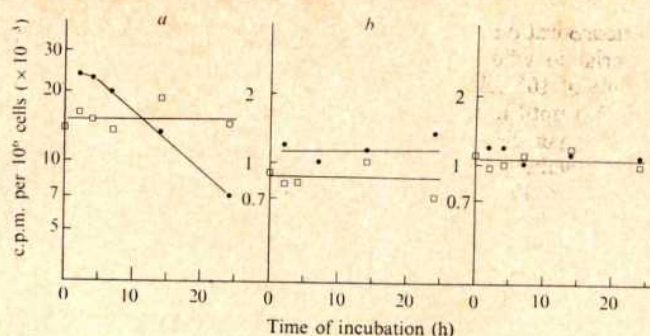


Fig. 2 Incorporation of labelled precursors by ts-546 cells. All the steps were carried out in warm rooms with temperature fluctuation controlled within $\pm 0.05^\circ$ C. 1×10^5 cells were inoculated into each 60-mm dish and incubated for 72 h at 33° C permitting the cells to reach log-phase growth at a concentration of about 1×10^6 cells per dish. At time 0, half of the dishes were transferred to 39° C. Two dishes each at the time of transfer and intervals thereafter were labelled with $0.5 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (a), $1 \mu\text{Ci ml}^{-1}$ ^3H -uridine (b) or $0.5 \mu\text{Ci ml}^{-1}$ ^3H -L-leucine (c) for 30 min at 33 and 39° C. The medium was then removed and 10% trichloroacetic acid added. Acid insoluble material was collected by filtration and radioactivity was counted in a Beckman scintillation counter. □, Cells at 33° C; ●, at 39° C.

Co.) without added antibiotics. To isolate the mutants, 33° C was used as the permissive temperature and 39° C the non-permissive temperature. Cells were treated with 10^{-5} M N-methyl-N'-nitro-N-nitrosoguanidine (NG) which killed approximately 70% of them. The rest were grown at 33° C and added to 60-mm Falcon plastic culture dishes at a concentration of 2×10^5 per dish and incubated at 39° C for 24 h, at which time $10 \mu\text{g ml}^{-1}$ cytosine arabinoside and $10 \mu\text{g ml}^{-1}$ 5-fluorodeoxyuridine were added. The dishes also received $40 \mu\text{g ml}^{-1}$ uridine to prevent 5-fluorouracil, a breakdown product of 5-fluorodeoxyuridine, from interfering with RNA synthesis¹¹. After a further 24 h at 39° C, the medium was changed to MEM and the dishes were returned to 33° C. When clones had developed, the cells were transferred to Linbro 24-well multi-dishes. Half the cells in each clone were added to wells in dishes incubated at 33° C and half were added to the corresponding wells in dishes incubated at 39° C. The cells which grew at 33° C but not at 39° C were removed and further tested for growth at 33° and 39° C. No mutants have been isolated without NG treatment. Of more than fifty mutants isolated, one, ts-546, was found in which mitosis is blocked in metaphase at the non-permissive temperature.

The wild-type cell differed slightly from the mutant in growth rate at 33° C. The parental HM-1 cells grew at 33° and 39° C with doubling times of 20 and 12 h respectively. Mutant ts-546 grew well at 33° C with a slightly longer doubling time of 25 h (Fig. 1). When the ts-546 mutant cells were placed at 39° C, the cell number increased slightly for 2-4 h and then declined (Fig. 1). The decrease in cell number was probably a result of the cell counting procedure used, in which medium was removed before trypsin was placed in the dishes to dislodge the cells attached to the bottom. The mutant ts-546 cells arrested in mitosis (described later) tend to be loosely attached or not attached and were readily removed with the medium. No cells remained attached to the dishes after 5 d.

When 50 or 100 cells were plated per 60-mm dish, the parental wild type cells plated with >90% efficiency at 33° and 39° C (Table 1). The plating efficiency of the mutant was slightly reduced at 33° C, but at 39° C, when up to 2×10^6 ts-546 cells were plated per 60-mm dish, no clone was found (Table 1). Cell killing at 39° C was thus independent of the density of cells inoculated. Of the 10^8 cells cultured at 39° C, two revertants have been found giving a reversion frequency of about 5×10^{-7} .

Incorporation of labelled precursors into acid-insoluble material in wild type and ts-546 cells was also measured. A sample of 10^5 cells was inoculated per 60-mm dish and grown for 72 h until log-phase growth was reached at approximately 10^6 cells per dish. Half the dishes were then switched to 39°C . At intervals, ^3H -thymidine, ^3H -uridine or ^3H -L-leucine was added for 30 min at both temperatures. Incorporation of ^3H -thymidine per cell began to decrease after 4 h for the cells incubated at 39°C (Fig. 2). The decrease could be partly or totally due to the fact that as the cells entered mitosis, they were arrested in metaphase (described later) and consequently unable to enter G1 and re-initiate DNA synthesis. No change in the incorporation of the labelled material into RNA or proteins was found at 39°C (Fig. 2). The incorporation procedure eliminated any loosely attached or floating cells when the medium was removed before trichloroacetic acid was added. This may explain why RNA synthesis per cell was not decreased even though a fraction of the cells was arrested in mitosis and expected to synthesise little or no RNA¹².

Mitotic figures were examined after the mutant cells were grown at 33°C and 39°C . When the mutant cells were grown at 33°C , normal mitotic figures were observed (Fig. 3a). After the cells were shifted to 39°C , many aberrant mitotic figures appeared (Fig. 3b). The chromosomes seemed no longer to be held together by the spindle. They were scattered as in colchicine-induced 'colchicine-metaphase'¹³ (C-metaphase), where spindle fibre mechanisms are partially or totally destroyed, and the chromosomes lose their normal orientation. To distinguish the figures seen here from C-metaphase, the aberrant mitotic

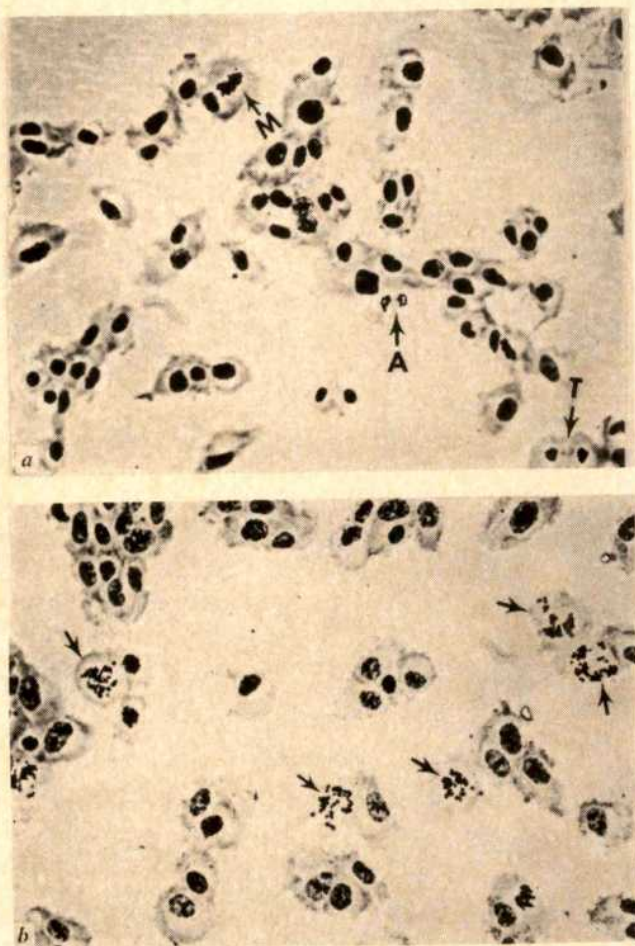


Fig. 3 ts-546 cells at 33°C . Cells grown at 33°C were washed with phosphate-buffered saline, fixed with 3:1 methanol-acetic acid and stained with aceto-orcein. Arrows point to normal metaphase (M), anaphase (A) and telophase (T) in (a) and aberrant ts-546-metaphases in (b). ($\times 200$.)

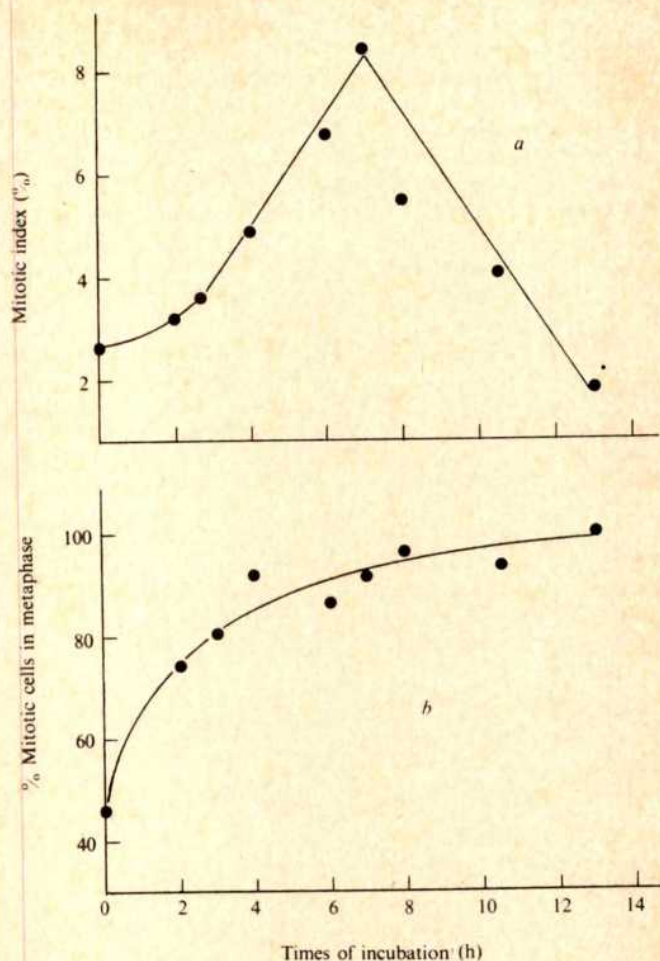


Fig. 4 Change in mitotic index and percentage of mitotic cells in metaphase in ts-546 cells after incubation at 39°C . 10^6 cells in log-phase growth per 60-mm dishes were transferred to 39°C at time 0. Cells were fixed and stained at intervals. The mitotic index (a) is expressed as the percentage of cells in mitosis. The percentage of mitotic cells in metaphase (b) is defined as

$$\frac{\text{No. of cells in metaphase and ts-546 metaphase}}{\text{No. of mitotic cells}} \times 100.$$

figures induced by temperature shift are termed 'ts-546-metaphase'.

Immediately after the cells were switched to 39°C , mitotic figures (metaphase, ts-546-metaphase, anaphase plus telophase) started to accumulate. The mitotic index, or the fraction of cells in mitosis, increased from approximately 3% to more than 8% after 7 h at 39°C and decreased thereafter (Fig. 4a). The fact that the mitotic index did not increase beyond 8% was most likely due to the fixation procedure with loosely attached or floating cells lost during washing. Time-lapse photomicro-

Table 1 Clone forming ability of parental (HM-1) and mutant (ts-546) cells at 33°C and 39°C

No. of cells plated	No. of clones formed			
	33°C		39°C	
	HM-1	ts-546	HM-1	ts-546
50	46.5	—	54.5	0
100	92.5	48.0	—	0
200	—	89.5	—	0
1,000	—	—	TMTC	0
10,000	—	—	TMTC	0
10^5	—	—	TMTC	0
2×10^6	—	—	TMTC	0

Cells were plated in 60-mm dishes and incubated. After 7 d at 39°C or 10 d at 33°C , cells were stained with haematoxylin and the clones counted.

TMTC, too many to count.

graphs demonstrated that, in a culture of ts-546 cells at 39° C, the number of round, refractile, mitotic-like cells increased continuously and subsequently floated into the medium. When a culture of ts-546 cells was incubated at 39° C for 24 h, at least 50% of the cells appeared to be rounded and mitotic-like. At the same time, few dumbbell-like figures characteristic of anaphase or telophase cells were seen (Fig. 5).

The number of metaphases and ts-metaphases increased from approximately 50% of the mitotic figures at the time of temperature switch to close to 100% 8 h later (Fig. 4b). The number of anaphases and telophases correspondingly decreased from 50% to nearly 0.

The results show that when hamster ts-546 mutant cells were switched from 33° to 39° C, the cells ceased to divide almost immediately. With further incubation at 39° C, cells were killed regardless of their density. Reversion frequency was about 5×10^{-7} . No generalised defect in DNA, RNA or protein synthesis was found at the non-permissive temperature. At 39° C the mitotic cells were arrested in metaphase and ts-metaphase, which is seen as a failure of the normal spindle fibre mechanism to hold the chromosomes in their normal metaphase orientation. The ts-metaphase figures, under a light microscope, appear to be similar to the c-metaphases resulting from colchicine treatment.

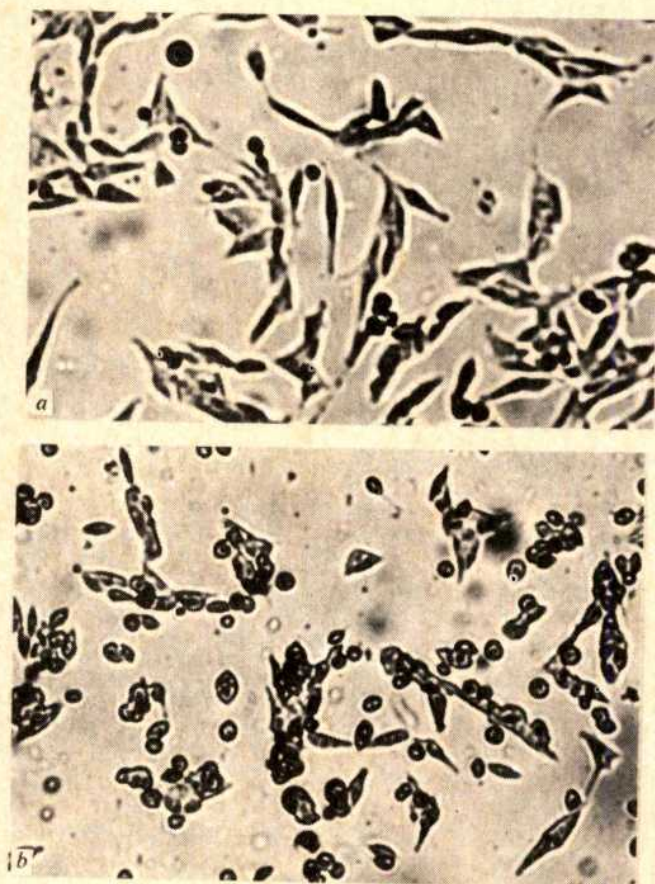


Fig. 5 Accumulation of mitotic-like cells at 39° C. Cells in dishes in log-phase grown at 33° C were incubated at 39° C for 24 h when the photograph was taken. ($\times 140$.)

Since mitotic cells generally synthesize very little or no RNA¹², one may ask that why is no reduction of RNA synthesis observed at 39° C? The cells used for measuring ³H-uridine incorporation were those which remained attached to the dishes where the maximum number of mitotic cells reached only 8% after shifting to 39° C (Fig. 4a). Consequently 92% or more of the cells used in the incorporation experiment were non-mitotic. The finding that the cells not in mitosis incorporate

a normal amount of ³H-uridine is thus compatible with the conclusion that the ts-546 cells in mitosis are blocked in metaphase at the non-permissive temperature.

Several mechanisms might account for the accumulation of mitotic cells in metaphase and ts-metaphase at 39° C. The gene controlling the production of spindle fibre proteins or microtubules could have suffered a mutation resulting in defectiveness and inability of the proteins or microtubules to orient properly at 38° C. A second possibility is that a protein holding the spindles together became defective. A third alternative is that a protein holds the spindle proteins and chromosomes together, becoming defective at the non-permissive temperature, so that the chromosomes are disengaged from the spindles. It is also conceivable that the defective protein, when normal, binds the spindles to the centriole. There is also the possibility that the synthesis of the spindle fibre proteins or other factors involved in karyokinesis failed to take place at 39° C. These possibilities are being investigated.

It is to be hoped that the isolation of temperature sensitive mutants defective in mitosis could lead to the identification of factors which are essential in mitosis. It might also be possible to identify the time during the cell cycle when these factors are synthesised. Furthermore, the possibility that mitosis can be arrested because specific factors become defective at a higher, non-permissive temperature may provide methods of arresting metaphase and controlling cell growth by specific inhibition of these factors.

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Facilitation of Learned Resistance to Audiogenic Seizures in Balb/cCrgl Mice by d-LSD-25

ALTHOUGH conditioning techniques applied to electrophysiological behaviour are presently being used in behaviour therapy for human epilepsy^{1,2}, complete cure is still a hope of the future. Extensive reports indicate that minute amounts of d-lysergic acid diethyl amide (d-LSD-25) could be used to assist current biofeedback training and other therapies used to effect change in the EEG and seizure susceptibility of

epileptics³ and we note many studies of the facilitation effect of small doses of *d*-LSD-25 on learning and memory⁴⁻¹⁸.

Several methods, including many behavioural ones¹⁴⁻¹⁸, are known for changing audiogenic seizure susceptibility in inbred mice. Elaboration of a conditioned response is known to interfere with the occurrence of these seizures¹⁹ and exploratory activity and learning ability are positively correlated with seizure resistance in rodents²⁰, as are several factors related to stress^{14,21}. We confirmed these effects in experiments with mice in which the administration of 3.65 μ g of *d*-LSD-25, 30 min before conditioning, significantly facilitated long-term habituation and also conditioned inhibition of audiogenic seizures induced by an intense, high-frequency sound presented 1 d after the end of training.

Long-term habituation was brought about by exposing the mice to a subseizure threshold tone 1-4 d before challenging them to seize with the louder tone. This effect is related to the classical 48 h refractory period found after intense stimulation¹⁴. Conditioned inhibition of seizures was accomplished by pairing a light with brief subthreshold tones, and subsequently presenting the light 5 s before the challenge stimulus on test night.

The use of multiple exposures to improve estimates of seizure susceptibility was first reported by Fuller and Sjursen²². Henry²³ later showed that a priming stimulation of only 30 s at an early age induced development of seizure susceptibility which peaked 4-5 d later, suggesting a method of more reliable control of seizure susceptibility. In standardising procedures for Balb/cCrgl mice, we found that peak seizure incidence (88%) on day 26 after priming on day 21 was equal to the cumulative seizure incidence computed as the percentage of mice that seized at least once during four consecutive exposures on days 21, 28, 35 and 42. In our experiments, the treatment variables were introduced between days 21 and 26.

Six hundred and eighty-five mice were randomly assigned to groups by split litter design, weighed and used to test the effects of pure *d*-LSD-25 (Sandoz) given intraperitoneally (7.3 μ g ml⁻¹; average dosage 180 μ g kg⁻¹; standard dose, 3.65 μ g per mouse) on the alteration of audiogenic seizure susceptibility by experimental and control procedures which are listed below. All mice were primed on day 21 (1900 h) and tested on day 26 (1900 h). The main experimental procedures were as follows. Series 1, running to escape from the hand of the experimenter in an activity wheel at 50 m d⁻¹ on days 22-26 (1900 h); series 2, the direct effect of *d*-LSD-25 given on days 21 and 26 (30 min before priming and testing respectively) as compared with the same injected only on days 24 and 25 (1900 h); series 3, the effect of *d*-LSD-25 given 30 min before training on long-term habituation on test night induced by five 1 min alternating exposures to a subseizure threshold 10 kHz pure tone (60 dB above 0.0002 dynes cm⁻² presented repeatedly on days 22-25 (1200 h) as compared with the effect when injected 30 min before receiving 20 min continuous exposure on days 24 and 25 (1200 h); series 4, the effect of *d*-LSD-25 injected 30 min before a 15 min non-random classical conditioning session of delayed light (25 W red, diffuse bulb)-tone (10 kHz at 60 dB above 0.0002 dynes cm⁻²) pairing (ISI, 1.5 s, US, 10 s, time-out, 15 s) on day 25 (1900 h). On day 26 the conditioned stimulus (red light) was presented 5 s before the challenge stimulus (condition CS in series 4 of Table 1).

Drug controls received equal amounts of handling and of the diluent (0.9% sterile saline) and yoke stimulation. Additional control groups were included as indicated in Table 1. For series 1, controls received equal amounts of exposure to the hand of the experimenter in a fixed activity wheel, in series 2, controls were not primed, and in series 3 they received equal exposure to the experimental chamber. For series 4, experiment 1, controls received equal cyclic exposure to light-only and tone-only. Controls in experiment 2 of series 4 received identical light-tone pairing but were not

Table 1 Effect of *d*-LSD-25 and learned resistance to audiogenic seizures in Balb/cCrgl mice

Series	Experiment	Condition	Drug	% Seized	N	adj. χ^2
Activity wheel training						
1		Run	—	62	37	
		Yoke	—	86	36	
		Total			73	4.60*
Direct action of LSD						
2		Primed	LSD	88	16	
			Saline	75	16	
		Unprimed	LSD	0	16	
			Saline	0	16	
		Total			64	0.59
	2	Primed	LSD	84	25	
			Saline	92	25	
		Total			50	0.09
Habituation and LSD						
3		Tone	LSD	50	50	
			Saline	84	50	
		Controls	LSD	90	10	
			Saline	90	10	
		Total			120	13.02†
	2	Tone	LSD	0	20	
			Saline	25	20	
		Controls	LSD	65	20	
			Saline	85	20	
		Total			80	27.73†
Conditioned inhibition and LSD†						
4		Light-tone	LSD	41	37	
			Saline	60	37	
		Light-only	LSD	66	37	
			Saline	66	37	
		Tone-only	LSD	60	25	
			Saline	60	25	
		Total			198	6.19
	2	CS	LSD	28	25	
			Saline	44	25	
		CS	LSD	52	25	
			Saline	56	25	
		Total			100	4.43

* $P < 0.05$.

† $P < 0.001$.

‡ LSD, light-tone treatment is significantly different from remaining control groups pooled in this series (adj. $\chi^2 = 5.31$, d.f. = 1, $P < 0.05$).

exposed to the conditioned stimulus on day 26 during challenging (Conditions CS).

All mice were primed and tested with a 90 s 10 kHz pure tone at 100 dB above 0.0002 dynes cm⁻². A concentration check after 6 months showed no change in the LSD solution kept in the dark, near room temperature.

The results are summarised in Table 1. Repeated locomotor practice significantly reduce seizure susceptibility by 24%. This effect is not strong and was not significant in a smaller sample (data included in experiment 1 of series 3) treated with *d*-LSD-25. The effect is not due to a single session of running just before testing on day 26. Earlier work with activity wheel stress and audiogenic seizures found very similar results¹⁵.

At 3.65 μ g per mouse, repeated or single doses of *d*-LSD-25 had no direct effect upon seizure susceptibility. Only one 3.65 μ g dose, however, increased conditioned inhibition and long-term habituation of audiogenic seizures in the presence of the conditioned-unconditioned and unconditioned stimulus respectively. The increase in habituation was more significant. Future work on conditioned inhibition might use more elaborate procedures to study the effects of *d*-LSD-25 on higher learning.

These results did not depend on *d*-LSD-25 being present during testing performed 24 h after injection and training. Controls showed learning occurring to a lesser degree independent of the *d*-LSD-25 state during acquisition. These results support earlier findings that low amounts of *d*-LSD-25 facilitate ongoing processes in the brain, affecting learning and memory⁴⁻¹³. Studies of the best time relationships between states and of the use of equal or preferably smaller doses to effect reverse tolerance are needed. The symptoms of overdose are well known to have effects opposite to those of lower doses⁹.

Mice exhibit the highest resistance to high-dose effects of *d*-LSD-25, as much as 300 μ g per mouse being required to produce overt changes in its behaviour²⁴. We have shown here that as little as 1% of this dose caused significant facilitation of learned behaviour which reduced subsequent seizure susceptibility. Although caution must be observed²⁵ these data may be extrapolated to the human condition on at least three grounds. Similar forms of reflex epilepsy exist between the two species (for example, startle, audiogenic and musicogenic epilepsy²⁶⁻²⁹). Acoustic stimulation has been found to be superior to intermittent photic stimulation or hyperventilation in defining the cortical paroxysmal focus in the EEG of humans with temporal lobe epilepsy³⁰. (There is reason to believe that a cortical focus facilitates the audiogenic seizure in mice.) Also, seizures in both species are subject to the effects of learning^{1-3,19}.

The facilitation effect of single low doses on learning has been reported for humans as well as several animal species in many different situations. Suggestions that *d*-LSD-25 causes undesirable side-effects, such as chromosomal damage, when used in clinically useful doses, have been refuted by investigations *in vitro* and *in vivo* in several species including humans^{3,31-37}. 'As neural degeneration and functional depression due to anticonvulsant medication are a problem³⁸⁻⁴⁰, human experimentation with low dose schedules and learning should be considered.

By comparing dose-effect data from the mouse and human it is possible to estimate the optimum dosage required to facilitate learning and memory in the human. The mouse detoxifies *d*-LSD-25 ten times faster, has a metabolic rate ten times greater and a much lower brain/body weight ratio than humans; so its high resistance to direct effects is not surprising. Overdose symptoms peak at approximately 300 μ g for the mouse²⁴ and 1,000 μ g for the human⁴¹. As 1% of this dose significantly reduced seizure susceptibility in mice through learning, the optimum dose facilitating condition inhibition of seizures in human epileptics could be as low as 10 μ g or less (that is, about 0.1 μ g kg⁻¹ as compared with the so-called moderate dosage of 1-2 μ g kg⁻¹). Further, since 100 μ g represents the anticonvulsant threshold in mice, at least 300 μ g should be required to suppress motor seizures directly in the human epileptic.

The physiological mechanisms underlying the effect of *d*-LSD-25 on brain function are relatively well understood⁸. It acts directly as an anticonvulsant when taken in high doses. Approximately 1-5 mg kg⁻¹ must be given to the mouse to increase seizure resistance independent of conditioning^{42,43}. This direct protection is related to the facilitation of motor and sympathetic nervous activity within this dose range for the mouse^{42,44-47}. High dose inhibition of synaptic transmission through the thalamus⁴⁸ is probably not a necessary part of the anticonvulsant action in mice, since all brain rostral to the inferior colliculus is unnecessary for elaboration of the audiogenic seizure sequence⁴⁹, but may be where subcortical activation of a cortical focus is necessary for the seizure to occur^{50,51}.

Bradley and coworkers⁵² showed that small amounts of *d*-LSD-25 amplify the ongoing effects of sensory collateral inputs to the reticular formation of the brainstem without directly affecting sensory input to the cortex⁵³ or directly exciting the output cell core⁵⁴ of the reticular formation. They later discovered that these effects focused attention of

the animal on to a neutral or habituated stimulus to the degree found with conditioned stimuli (where response thresholds were not affected by the dosage used) without directly arousing the animal or preventing its sleep. They suggested that in small amounts, *d*-LSD-25 increases the significance level of sensory stimuli by facilitating mechanisms which underlie attention and block short-term habituation. The inhibitory effects of attentional mechanisms in animal and human epileptics are extensively documented although they are still not fully understood⁵⁵.

Amphetamine has also been shown to facilitate learning and memory only within an optimal dose range⁵⁶ and also to reduce audiogenic seizure susceptibility at high doses^{31,42,43}. An optimal range in the dose-effect curve is a classical finding. Some drugs that facilitate learning at low doses, however, act as poisons and convulsants at higher ones (for example, strychnine)⁵⁶. Drugs chosen to facilitate behaviour therapy for epilepsy could conveniently act as anticonvulsants at high or cumulative doses while simultaneously minimising side-effects.

A recent review⁵⁷ re-affirmed past conclusions that LSD can be a safe therapeutic instrument when properly administered to suitable subjects in controlled settings. Tetrahydrocannabinol^{58,59,60} and psilocybin^{50,51} also act as anticonvulsants. This combination of facilitation of learning and memory in minute doses, direct anticonvulsant action in the upper dose range, reverse tolerance with proper scheduling, and the absence of addiction and proven side effects directly attributable to the drugs, suggests the use of psychedelic compounds as effective and safe adjuncts to current behavioural and electrophysiological biofeedback procedures presently used to treat epilepsy and a variety of other illnesses.

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Augmentation of Cytotoxic Drug Action by Antibodies Directed at Cell Surface

THE concept of attaching cytotoxic agents to tumour-specific antibodies in the treatment of cancer is therapeutically attractive. In this way the specificity of the agents would be increased and their systemic toxicity reduced. This immunochemotherapeutic approach has been successfully applied in experimental systems when the cytotoxic agent has been covalently bound to the antibody¹⁻³. Similar success has been reported with the nitrogen mustard chlorambucil, merely physically adsorbed on to the globulin fraction from tumour-specific antiserum

without the actual formation of a covalent bond⁴. It is surprising that antibody could act as a carrier for chlorambucil under these conditions, as has been proposed⁵, as the two molecules would be expected to dissociate in the *in vivo* environment. It is, therefore, probable that the increased cytotoxicity of chlorambucil in the presence of antibody is attributable to some other mechanism. The experiments described here are an attempt to elucidate this problem further.

The target cells for these experiments were polyoma-transformed BHK21/C13 cells (J₁) grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum which had been heated to 56° C for 30 min to inactivate complement. The cells were collected using a Tris-buffered saline containing 0.02% versene. An antiserum to these cells was produced in New Zealand white rabbits. Each rabbit received approximately 1×10^8 intact cells intramuscularly twice weekly for 4 weeks. The resulting antiserum contained antibodies for surface components of J₁ cells as shown by membrane immunofluorescence by the indirect method using fluorescein-labelled sheep antirabbit globulin (Miles-Serevac). The antiserum was also cytotoxic for J₁ cells in the presence of guinea pig complement (Wellcome) as shown by isotope release from cells prelabelled with radioactive chromium (⁵¹Cr); 50% of cells were lysed at a 1:320 dilution. Control normal rabbit serum (NRS) was obtained by bleeding the rabbits before immunisation. Both the NRS and the immune serum (IRS) were heat inactivated, sterilised through a 0.22 μ Millipore filter and stored

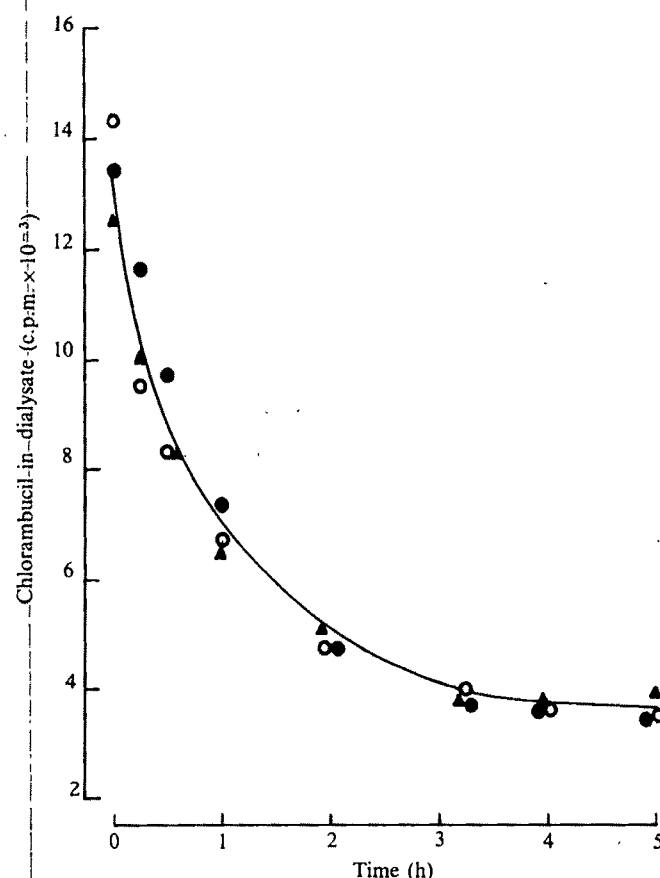


Fig. 1 10 ml volumes of undiluted calf serum were placed in lengths of Visking dialysis tubing (0.25 inch diameter), which were then arranged as flat coils and covered with 10 ml of one of the following solutions: (1) 1×10^{-6} M ³H-chlorambucil (specific activity 118 mCi mmol⁻¹) in PBS (○); (2) rabbit immunoglobulin (IgG) (Miles-Serevac) 50 μ g ml⁻¹ with 1×10^{-6} M ³H-chlorambucil preincubated on ice for 15 min (▲); (3) bovine serum albumin 50 μ g ml⁻¹ with 1×10^{-6} M ³H-chlorambucil preincubated on ice for 15 min (●). Dialysis proceeded at 37° C and 100 μ l samples of the dialysate were taken at timed intervals for scintillation counting.

at -20°C until used. Chlorambucil BP (Wellcome) was prepared just before use by dissolving a few mg in 0.5 ml of 0.5 M sodium bicarbonate, at room temperature and then immediately diluting in cold phosphate-buffered saline solution (PBS), pH 7.2.

the possibility that the increased sensitivity of the cells to chlorambucil was due to an increased rate of mitosis; (3) since it has been shown that chlorambucil in high concentrations ($6 \times 10^{-3}\text{ M}$) causes lysis of red blood cells⁷, the possibility that IRS may allow lysis of J_1 cells by lower concentrations of

Table 1 Cloning efficiency of Py BHK(J_1) cells treated with combinations of C, NRS and IRS described in text

Means \pm standard deviations of colonies in quadruplicate cultures.									
PBS	C	NRS	NRS/C	NRS-C	NRS+C	IRS	IRS/C	IRS-C	IRS+C
50 ± 2.2	38 ± 5.7	64 ± 6.8	39 ± 6.1	59 ± 8.7	39 ± 10	53 ± 16.5	14 ± 7.8	58 ± 6.8	12 ± 11

Differences: NRS/C and IRS/C, $P < 0.01$; NRS+C and IRS+C, $P < 0.02$; IRS/C and IRS+C, not significant.

The effect of chlorambucil on J_1 cells in the presence of immune serum was studied using a colony formation inhibition assay. One hundred cells were plated in 50 mm plastic Petri dishes in a 4.5 ml volume of medium with 10% inactivated calf serum. Then the cultures received one of the following additions: (1) 0.5 ml of PBS alone or containing one of the following substances: chlorambucil (C) to give a final concentration in the culture medium of $6 \times 10^{-6}\text{ M}$, or either NRS or IRS at a final dilution of 1 : 80 in the culture; (2) 0.5 ml of PBS with NRS or IRS mixed with C in the cold so that only physical adsorption of the drug to protein but little or no covalent reactions would occur⁶ (NRS/C and IRS/C); (3) 0.5 ml of PBS with NRS or IRS mixed with C and preincubated at 37°C for 24 h to allow complete reaction of the C, mainly alkylation of protein but also some hydrolysis⁶ (NRS-C and IRS-C); (4) NRS or IRS in 0.25 ml of PBS and C in 0.25 ml PBS added at the same time but separately to the dishes (NRS+C and IRS+C). In all cases the final concentration of chlorambucil and the dilution of NRS or IRS was the same. After incubating the dishes in humidified incubators flushed with carbon dioxide for 8 d, the medium was aspirated, the colonies were washed once with PBS, fixed with methanol, stained with 20% Giemsa stain and counted.

The results (Table 1) show that the cloning efficiency was the same with PBS, NRS alone, IRS alone, NRS-C and IRS-C. In those dishes that received active chlorambucil, namely C, NRS/C and NRS+C, there was a small reduction in the number of colonies. The reduction was however much greater when the drug was in the presence of IRS (IRS/C and IRS+C). Preincubation of IRS and chlorambucil in order to favour the adsorption of the drug on to antibody was not a prerequisite for augmenting the cytostatic effect of the drug.

These results corroborate, in a different system, those of Ghose *et al.*⁵ but do not support the hypothesis that they occur because the antibody acts as a carrier for chlorambucil. In fact, the identity of the effect of IRS/C and IRS+C would then require that chlorambucil associates preferentially with and binds strongly to rabbit immunoglobulins in competition with the calf serum proteins present in the medium. That this is not the case was shown by the experiment described in Fig. 1. The results show that the rate of disappearance of chlorambucil from the dialysate is the same whether or not it contains rabbit immunoglobulin, thus excluding preferential binding of chlorambucil to the immunoglobulin.

The mechanism whereby antibodies directed at the cell surface increase the susceptibility of polyoma-transformed BHK21/C13 cells (J_1) to the cytostatic effect of chlorambucil remains unknown. Several possibilities were ruled out by the following results. (1) The uptake of tritiated-chlorambucil by IRS-treated cells was not different from that of NRS-treated cells; (2) the mitotic rate of cells in the presence of IRS or NRS as determined by growth curves was the same, thus excluding

chlorambucil was considered. Cells were prelabelled with ^{51}Cr , treated with IRS or NRS together with graded doses of chlorambucil, and ^{51}Cr release was measured. While it was confirmed that these cells were lysed by $6 \times 10^{-3}\text{ M}$ chlorambucil too, there was no lysis in lower concentrations with either IRS or NRS.

Experiments using other cell systems and agents are now in progress to test the generality of this augmenting effect of antibodies on cytotoxic drug action and its possible use in cancer therapy. It is known that when tumours are associated with a well developed humoral immune response, for example in Burkitt's lymphoma⁸ and choriocarcinoma⁹, the response to cytotoxic drugs is often dramatic. We speculate on the basis of the experiments described above that in these malignant diseases it may be the binding of antibody to the tumour cells that by some means, not yet determined, is responsible for their marked susceptibility to chemotherapy.

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Spatial Heterogeneity and Population Stability

THE ability of populations to absorb perturbations is often investigated theoretically by some variant on the prey-predator, Lotka-Volterra, equations. Usually the treatment is in terms of linearised equations and thus deals with very small perturbations from a steady state. Recently, May¹ has enlarged the scope of investigation by considering perturbations from limit cycles rather than divergence from the simple steady state. This theoretical approach assumes populations uniform in space if not in time, but much of the variability found in nature is not only in large temporal cycles but in smaller-scale spatial patchiness. This variability may be handled statistically to give, for some specified area, a mean and variance for the populations. These can be used for 'sensitivity analysis' of the output from a theory or computer model, or as a basis for stochastic treatment. In either case this variability, as a function of changes in space, is not an intrinsic part of the model. Yet much experimental work shows that populations of prey or predator with limited abilities to disperse can produce spatially heterogeneous patterns, and the scale and complexity of these patterns may have some effect on persistence of these populations^{2,3}.

The technical problem in theorising about the consequences of dispersal lies in quantifying the rate of dispersal. In the sea, this can be overcome for planktonic populations such as phytoplankton and small herbivorous copepods which are almost entirely at the mercy of water movement and yet display considerable patchiness⁴. The dominant form of motion in the open sea away from areas of strong ocean currents is turbulent lateral diffusion⁵. This diffusion is similar, mathematically, to ordinary Fickian diffusion although on a much larger scale. To study the effect of this process on populations and to include the non-linear effects, it is simplest to begin with an expansion of the Lotka-Volterra equations to include variation along one spatial dimension, x . For phytoplankton, P , and herbivorous copepods, H , the interactions can be expressed as

$$\begin{aligned}\partial P/\partial t &= aP - bPH + \partial/\partial x \kappa \partial P/\partial x \\ \partial H/\partial t &= cPH - dH + \partial/\partial x \kappa \partial H/\partial x\end{aligned}$$

Assume that at some time there are random perturbations within the limits $(0, L)$ of x , expressed as Fourier series,

$$P = \sum_{n=0}^{\infty} A_n \cos \lambda_n x, \quad H = \sum_{n=0}^{\infty} B_n \cos \lambda_n x, \quad \lambda_n = 2\pi n/L$$

then the time rates of change of the coefficients A_n and B_n are expressible in the form,

$$\begin{aligned}dA_n/dt &= A_n(a - \lambda^2 n^2 \kappa) - bC_n \\ dB_n/dt &= cC_n - B_n(a + \lambda^2 n^2 \kappa)\end{aligned}$$

where

$$C_n = 1/2(\sum_{r+s=n} A_r B_s + \sum_{r-s=n} A_r B_s)$$

The terms involving C_n arise from the non-linear interactions of P and H . When these are ignored, as in the linearised study of small disturbances, or when only one population is considered, then perturbations at any wavelength are either amplified or damped at that wavelength and it can be shown⁶ that perturbations below a certain critical wavelength will be damped out by the processes of diffusion. In other words, only large patches would be expected to persist against the smoothing effects of turbulence.

The introduction of the non-linear terms leads to entirely different conclusions. Not only do perturbations interact to cause shorter wavelength 'harmonics' (the first term in C_n) but also they interact to give 'beat frequencies' at longer wavelengths (the second term in C_n). In particular, the perturba-

tions alter the mean. For example, for the mean value A_0 of P in $(0, L)$,

$$dA_0/dt = aA_0 - bA_0B_0 - 1/2b \sum_{r=1}^{\infty} A_r B_r$$

The first two terms on the right-hand side correspond to the normal 'Lotka-Volterra' equation⁷ which produce bounded oscillations with time. The spatial variations A_r, B_r ($r \geq 1$), however, interact to alter this mean. Thus, perturbations at any wavelength can, in theory, act to displace the prey and predator from their neutral limit cycle. In this way small-scale spatial variability in the populations which, on the linearised theory, would be damped out could, by directly displacing the mean, lead to a random walk to extinction as happens with stochastic effects on the ordinary Lotka-Volterra equations⁸ without diffusion.

As it would seem that diffusion or dispersal can never be sufficient to damp out fluctuations, these theoretical effects emphasise the need for positive stabilising factors, such as functional responses⁹ acting at the average population levels. The conclusions also imply that, when considering the effects of variability within an ecosystem, it is inadequate to introduce these by studying the effects of variation of each population on a model of the average population levels of an ecosystem. This ignores the interactions which can occur spatially and which thereby introduce changes in these average levels. Although the very simple, Lotka-Volterra, equations have been used here, more complex reactions, which introduce stabilising functions but do not consider spatial changes, should still have the same inadequacies in determining the limits of stable response in the face of environmental fluctuations.

These concepts have been developed for marine planktonic communities but the principles involved should apply to populations where dispersal is a significant feature of their life cycles. For plankton it is possible to quantify the effects on stability introduced by the combination of diffusion and spatial heterogeneity and this will be described elsewhere.

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Energy cost of gliding flight in herring gulls

By using the interplay of aerodynamic and gravitational forces, birds are known to travel considerable distances on fixed wings. The aerodynamics of this form of flight has been studied in the field¹⁻³ and in wind tunnels⁴⁻⁶, but the energetic cost is unknown. We report here experimental determinations of oxygen consumption during prolonged gliding.

Wild-trapped herring gulls (*Larus argentatus*) were trained to fly in a wind tunnel⁵ while wearing light-weight (10 g) ventilated head masks. From an initial sample of eight animals, only two would learn to glide consistently

TABLE 1 Metabolic rates during gliding flight in two herring gulls (calculated from oxygen consumption, using $1 \text{ l O}_2 \text{ h}^{-1} = 5.58 \text{ W}$)

Gull No.	Mass (kg)	No. of flights	Resting	Metabolic rate (W)			
				Mean	Gliding	Minimum	Mean increase
1	0.965	5	6.64 ± 0.58	12.50 ± 1.60		9.95	$1.9 \times$
2	0.859	4	6.57 ± 0.92	15.41 ± 0.81		14.71	$2.4 \times$

Values are means \pm standard deviation.

for periods of twenty minutes without flapping. All the reported data are from these two animals. Oxygen consumption was measured by drawing room air through the mask at a metered rate of 25 l min^{-1} and measuring the difference in oxygen concentration of the gas from the mask and that in room air (for details, see ref. 8). Oxygen consumption was taken as the stable minimum value maintained for at least 5 min of the 20 min test. We assume that this stable value represents steady state oxygen consumption as no increase was apparent at the end of the gliding periods. Resting measurements were made both before and after glides on birds standing unrestrained on the floor of the wind tunnel under the same conditions of temperature and illumination. Stable minima maintained over 20 min periods were taken as resting oxygen consumption levels. Food was withheld from the birds for 16 h prior to all experiments and in all metabolic calculations, R.Q. was taken as 0.8. Air speed and glide angle were adjusted to values at which the bird had minimum acceleration relative to the tunnel. For both animals this resulted in an air speed of 10.8 m s^{-1} and a glide angle of 6.5° below horizontal. The air temperature was 27.5 to 29.2° C .

Table 1 shows results from resting and gliding birds. Oxygen consumption at rest for both birds was about 1.7 times the expected standard rate as calculated from the allometric equation of Lasiewski and Dawson⁷. For one bird, gliding resulted in a mean increase in oxygen consumption to 1.9 times the resting level and for the second bird to 2.4 times the resting level.

It is interesting to compare our measurements with those taken for steady state flapping flight under similar wind tunnel conditions. Several such determinations have given an oxygen consumption during steady state horizontal flapping flight of about seven times the resting level⁸⁻¹⁰ (this ratio will probably vary as additional species are studied). Another comparison is with Pennycuik's theoretical calculations of the cost of gliding². By adding the calculated standard metabolic rate to an estimate of the power input by the pectoral muscles during gliding, he arrived at a figure of about 1.5 times the basal rate for the white-backed vulture. This provides an interesting comparison with our minimum value of 1.5 times the resting level (compare Table 1).

We conclude that gliding flight represents an increase to about twice the resting level of oxygen consumption and has a substantial energy-saving advantage over flapping flight.

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Evidence Against a Genetical Component to Performance on IQ Tests

Eaves and Jinks¹ have failed to note the significance of Scarr-Salapatek's² data, which provides important evidence against the heritability of IQ performance. A straightforward overall evaluation of this study, together with a review of evidence³⁻⁷ which Eaves and Jinks consider to be more secure in establishing a genetical component to IQ performance shows that: (1) the upper limit of IQ heritability in Scarr-Salapatek's study is $15\% \pm 16\%$. This is consistent with zero heritability and directly contradicts the higher figures claimed by other studies³⁻⁷; (2) other studies⁵⁻⁷ which use identical-fraternal comparisons to derive apparently higher upper limits to heritability do not take into account the more similar treatment frequently given to identical twins. When this is adjusted for, these studies are consistent with low or zero heritability; (3) the similarity between separated identical twins, used by Jinks and Fulker³ to derive an 80% heritability estimate, can be quantitatively accounted for by highly correlated placement (ref. 8 and L. J. Kamin, Invited Address, Eastern Psychological Association, Washington DC, March 1973) with little or no genetical component.

We use the methods of Jinks and Fulker for our evaluation. The difference in correlation coefficients for identical twins raised together (MZ) and fraternal twins raised together (DZ) is

$$r_{MZ} - r_{DZ} = [G_1 + E_1(DZ) - E_1(MZ)] / \sigma_T^2 \quad (1)$$

where

$$\sigma_T^2(DZ) = \sigma_T^2(MZ) \text{ and } G = G_1 + G_2$$

In general $E_1(DZ) > E_1(MZ)$ since identical twins are treated more similarly than fraternal twins. Identical twins are of the same sex, are frequently dressed alike, given the same toys and mistaken for one another. Thus, large differences in correlation of identical and fraternal twins do not necessarily mean high heritability, or any heritability^{4,8}, and studies of MZ-DZ differences can only give upper bound estimates for G_1

$$G_1 / \sigma_T^2 \leq r_{MZ} - r_{DZ}$$

To obtain the upper limit heritability from Scarr-Salapatek's data we have, for the error in z scores of MZ twins, $\sigma_{MZ}^2 = a\sigma_{\text{res}}^2 + b\sigma_{\text{os}}^2$. The coefficients a and b are given in Eaves and Jinks¹. With $\sigma_{\text{res}}^2 = 1/(N_{\text{ss}} - 3/2)$ (ref. 9) where N_{ss} is the number of same sex pairs and similarly for σ_{os}^2 and with $\sigma_r = (1 - r^2)\sigma_z$ we compute the standard deviation of the correlation coefficient r_{MZ} . Assuming $Z_{DZ} = Z_{\text{os}}$ (this raises

the heritability estimate since in general same sex DZ twins are treated more similarly than opposite sex DZ twins) we then compute the variance, σ_d^2 , of the difference, $r_{MZ} - r_{DZ}$, $\sigma_d^2 = \sigma_{MZ}^2 + \sigma_{DZ}^2 + 2(1 - r_{MZ}^2)(1 - r_{DZ}^2)(P/(1 - 2p))\sigma_{os}^2$ where $p = 0.30$ for whites and $p = 0.34$ for blacks. For Scarr-Salapatek's Table 8 we obtain four independent estimates of the difference $r_{MZ} - r_{DZ}$ as follows: black, lower socio-economic status (SES) = -0.165 ± 0.185 ; blacks higher SES = 0.109 ± 0.184 ; whites, lower SES = -0.046 ± 0.340 ; whites, higher SES = 0.160 ± 0.110 . Using least squares the overall best fit is

$$r_{MZ} - r_{DZ} = 0.075 \pm 0.082 \quad \chi^2 = 2.4(3 \text{ d.f.})$$

or

$$G_1 \leq 7.5\% \pm 8.2\%$$

Estimating the heritability depends on the relative magnitudes of G_1 and G_2 . Previous work has used $G_1 = kG_2$ with $k \approx 1$ (refs 1, 2, 5, 6). For purposes of comparison we choose $k = 1$ and obtain

$$h^2 \leq 15 \pm 16\%$$

which is a new upper limit to the heritable component to performance on IQ tests.

This result is to be compared with other estimates⁵⁻⁷ cited by Eaves and Jinks derived from the identical approach. It is 4.0 standard deviations lower than the figure of $h^2 = 80\%$ quoted by Jensen^{5,6} and promulgated in popular accounts¹⁰. The probability of a discrepancy this large occurring by chance is less than 10^{-4} if the true heritability is $h^2 = 80\%$.

We fail to understand why Eaves and Jinks are prepared to discard this result. To do so only perpetuates the apparently common practice in this field of ignoring or failing to report evidence against the genetic hypothesis. As Scarr-Salapatek describes it "there are few published reports of null results unless a major theoretical point is at issue. I, for one, obtained the same correlation (0.61) for blood-grouped MZ and DZ twins on an individually administered test of non-verbal IQ and did not submit the results for publication (because no one would believe that MZ twins were not more similar, there were only sixty pairs and so on)"¹¹.

The apparent discrepancy between $h^2 \leq 15 \pm 16\%$ and the higher figures reported elsewhere^{5,6} is resolved by estimating the size of the 'treatment' effect, caused by the more similar treatment of MZ twins. By comparing the correlations of groups with the same G_1 we obtain pure treatment effects of expected magnitude less than $E_1(DZ) - E_1(MZ)$. From Victorin¹² $\Delta r_{DZ}(\text{male-female}) = 0.12 \pm 0.05$ and $\Delta r_{MZ}(\text{male-female}) = 0.15 \pm 0.09$. (For comparison $\Delta r(\text{males})(MZ-DZ) = 0.18 \pm 0.09$ and $\Delta r(\text{females})(MZ-DZ) = 0.15 \pm 0.05$ in the same data). Similarly from Huntley¹³ $\Delta r_{DZ}(\text{same sex-opposite sex}) = 0.21 \pm 0.09$ and from Jinks and Fulker³ $\Delta r_{MZ}(\text{boys-girls}) = 0.17 \pm 0.16$. From Erlenmeyer, Kimmeling and Jarvik⁷ we see that the range in correlations for studies of the same groups is large. For parent-child $\Delta r = 0.6$, for siblings $\Delta r = 0.5$, and for same sex DZ twins $\Delta r = 0.45$. Since in Jensen's method⁶ $\Delta r(MZ-DZ) \sim 0.35$ yields 70% heritability, these figures demonstrate that the treatment effect acting alone is enough to produce the Δr s observed between MZ and DZ twins.

We now consider the four studies of separated twins. Kamin has demonstrated gross errors in methodology and analysis in these studies but the Shield's data is still useful for a quantitative comparison of genetic and environmental models. Jinks and Fulker³ analyse this data in terms of the components G_1 , G_2 , E_1 , E_2 , and $G = G_1 + G_2$. Naming a variance component G , however, does not mean that it is genetic. A model with the substitutions $E_T \leftrightarrow E_1$, $E_F \leftrightarrow E_2$, $E_A \leftrightarrow G_1$, $E_S(DZ) \leftrightarrow G_2$ and $E_S(MZ) \leftrightarrow G$ is an environmental model formally identical to the simple genetic model where the sources of variance are: E_T , differences in treatment of identical twins; E_A , additional differences due to the different appearance of DZ twins; E_F , between pairs family differences; E_S , differences in social environment. The results are shown in Table 1.

The large value of $E_S(MZ)$ means there is a high degree of similarity in the environments of separated MZ twins. Such

TABLE 1 Comparison of Variances of Environmental and Genetic Models Based on Analysis of Shield's Data

Environmental model		Genetic model
Variance due to differences in Social environment ($E_S(MZ)$)	71% \pm 12%	Genetics (G)
Family (E_F)	0%	Family and social environment (E_2)
Within family treatment (E_T)	29% \pm 12%	Within family treatment (E_1)

a conclusion is born out by Fehr's⁸ and Kamin's analysis of Shield's data. The null E_2 in the genetic model contradicts the known fact that social environments have a large effect on IQ performance¹⁴ while the null E_F in the environmental model means that differences in the way families in the same social environment treat children have little effect on IQ performance compared to differences in the way the social environment treats children. The environmental model is actually more consistent with the data than the conventional genetic model.

In summary, we note the following. The upper limit obtained from Scarr-Salapatek's data of $h^2 \leq 15\% \pm 16\%$ ($G_1 \leq 7.5 \pm 8.2\%$) is evidence against the heritability of IQ performance. Other low results go unreported¹¹ and this lends additional weight to her results. This 15% figure and every higher estimate reported in MZ-DZ studies contains an uncontrolled environmental component deriving from the more similar treatment accorded identical twins. Estimates indicate that this effect is large enough to make other upper limits derived from MZ-DZ comparisons consistent with zero. The separated twin studies when properly analysed also give heritability estimates which are low or consistent with zero.

We conclude that the evidence used to support high heritability of IQ performance actually yields low estimates of heritability consistent with zero and not larger than the upper limit of $h^2 \leq 15\% \pm 16\%$. Zero heritability of IQ performance means that the individuals in the population all have the same genetic potential for the expression of this trait.

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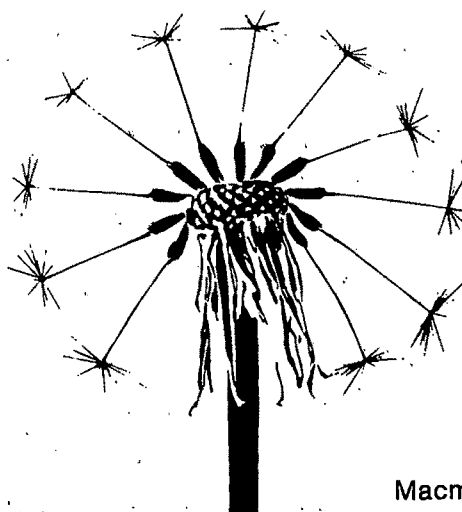
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book reviews

Watch without Mother

Separation: Anxiety and Anger. Vol. 2 of Attachment and Loss. By John Bowlby. Pp. xviii+444. (The International Psychoanalytical Library No. 95.) (Hogarth and the Institute of Psycho-Analysis: London, May 1973.) £4.50.

PSYCHOANALYSIS has been long on theory and short on experimental evidence. Dr John Bowlby, the psychoanalyst best known for his work on the effects of maternal deprivation on children, has dedicated his later career to remedying that gap. He, and the institution with which he is connected, the Tavistock Institute of Human Relations, are now trying to use the techniques of scientific research to study the way in which the human personality develops. (The institute's director, Dr Dorothy Heard, was originally trained in the biosciences.) This volume is the second of a projected trilogy in which Dr Bowlby, looking at his basic theory that the loss or prolonged absence of the mother harms the child, asks why and how?

His method is to take as primary data the observation of young children in defined situations, and then to extrapolate forwards. He sees this as a reversal of the traditional direction of psychoanalytic hypothesis-making, which is to confront a fully formed personality and then to conjecture how it got that way. Nonetheless, Dr Bowlby wants it clear that his frame of reference is still psychoanalytic. The concepts which he sees as basic to the growth of personality are separation anxiety, defence, the bonds between people and so forth which, in his view, have been ignored by the behavioural sciences apart from psychoanalysis. But he is adamantly not a classical Freudian. Instead, he has a "perspective based on a Darwinian type theory of evolution." What this means in practical terms is that he is willing to use animal behaviour as evidence and in theoretical terms that he sees certain kinds of fears as functional, of survival value to the human race, rather than as infantile or neurotic.

Bowlby's exposition, however, is more interesting than his conclusions. From assembled evidence, he shows that the strong reaction to a mother's absence is shown by babies from the age of 26 weeks onward. He analyses the findings that a single separation of 6 days or more from its mother is harmful to an infant rhesus monkey, with effects observable as much as 2 years later. ("The

only safe dose", he concludes about maternal deprivation in these monkeys, "is a zero dose.") He declares unqualifiedly that while few other predictions can be made about children separated from their parents, it is clear that when a child is threatened with being abandoned (either explicitly or obliquely, by over-hearing quarrels), the effects of an actual separation when it occurs are likely to be magnified and to persist.

He also feels on sure ground when he rejects some cherished ideas—Freud's hypothesis of 1926 that the fear of mother's absence results from the infant's learning that when she is absent, his physiological needs go unmet, and the belief held by Freud and almost everybody else that an excess of parental affection can make a clinging child. The evidence is all the other way, he says, with documentation, that overdependence is the result of bitter experience, not excess of gratification. So why does the unwilling separation from its mother or her substitute make a child respond with fear? Bowlby offers tentative conclusions and calls for more research. His hypotheses are (1) that such fear may be instinctive, (2) the infant may learn to associate its mother's absence with distress just as it learns to associate her presence with comfort and (3) an infant may learn that it is more afraid of fear-arousing situations, such as a loud noise, when its mother is absent and thus associate her absence with fear.

Bowlby once again offers a more kindly, less punitive approach to the child than is generally taken, either by the post-Victorians or the Freudians. He deplores the fact that most people still believe that a normal child should not make a fuss when its mother leaves it at school and that "babyishness" is something to be corrected. Or that something as positive as parental love for a child could cause the pathology of overdependence.

Dr Bowlby's words are so influential—he kept a whole generation of mothers home from work—that they have to be weighed not only in the light of their contribution to psychoanalytical theory but of what will be made of them by the public. The law, the schools and many parents still have scarcely a glimmer of perception that anxiety in children cannot be cured by preaching self-control. Dr Bowlby's humane—and rational—explanations of the origins of the child's anxiety need to be listened to. But at the same time he does raise

spectres of smothering, of the claustrophobic nuclear family of which Dr Edmund Leach warned in Reith Lectures a few years ago. Is a zero dose of maternal deprivation a good thing?

BRENDA MADDOX

Ethereal molecules

Molecules in the galactic environment. Edited by M. A. Gordon and L. E. Snyder. Pp. xv + 475. (Wiley: New York and London, October 1973.) £9.50.

"ALL of the elements that make up our bodies (with the exception perhaps of hydrogen) have been formed in stars. Thus each and every one of us is, in a very real and literal sense, a little bit of stardust." These words of Willy Fowler give the clue to the wide current interest in interstellar molecules for it is believed that they may be an important link in the evolution of life in the Universe. Of course even the largest molecules— $\text{CH}_3\text{C}_2\text{H}$ (methylacetylene); HCOCH_3 (acetaldehyde)—found by radio searches in the interstellar medium are a long way from the truly complex molecules which characterise living organisms. Nevertheless, since molecule synthesis must begin somewhere, it is gratifying that the first steps of this synthesis are amenable to investigation in the depths of interstellar space even if later steps are forever lost in the uncharted atmosphere or sea of some early planet. But who really knows? Ten years ago nobody would have predicted that such a rich field of interstellar chemistry lay around the corner—a veritable Black Cloud just waiting to be discovered.

In November 1971 a conference to discuss recent discoveries was held at the National Radio Astronomy Observatory in Charlottesville, USA. This book is the conference report edited by two astronomers who have been in the forefront of the radio discoveries.

In addition to the biological implications another important aspect of recent work is the interstellar chemistry of molecule formation (and destruction). Available observations give crucial information about densities, temperatures and excitation conditions in the molecule-bearing clouds. These allow some appraisal of the proposed theories of molecule formation. It is clear that a variety of processes are at work—some are surface reactions on dust grains and others are gas-phase reactions of varying complexity, but we are still far from a complete understanding of all the proc-

esses at work in interstellar chemistry. There is no indication of how complex the undiscovered interstellar molecules may be. Even so it is unlikely that DNA will ever be discovered in interstellar space.

One field of study scarcely touched upon in this volume is the use of molecular observations as probes of the interstellar medium. They have proved particularly significant in studies of dense gas clouds such as those found in the general interstellar medium, in the immediate vicinity of certain recent formed stars and in the galactic centre region.

In summary, this book provides a number of excellent review articles—especially those of Field, Iben, Litvak and Ponnampertuma, and a medley of research reports. It will be read with interest by astronomers and biologists.

R. D. DAVIES

Strong probability

Stochastic Analysis: A Tribute to the Memory of Rollo Davidson. Edited by D. G. Kendall and E. F. Harding. (Wiley Series in Probability and Mathematical Statistics.) Pp. xiii + 463. (Wiley: London and New York, August 1973.) £11.

NAMES of branches of science are often coined to express an insight into their nature, and 'stochastic analysis' is one of these. It witnesses to the end of the isolation of probability theory, to its penetration into, and its complementary illumination by, many different areas of pure and applied mathematics. Today one finds probabilistic methods cropping up in the most surprising places, just as probability theory itself now calls on mathematical tools of every variety.

Twelve years ago the University of Cambridge established a chair of mathematical statistics, and shortly afterwards moved its Statistical Laboratory from the basement of the chemical laboratories to a disused paper warehouse. In such unpromising surroundings there grew up the leading centre in Britain for research in stochastic analysis, attracting visitors from all over the world. This volume, which is to be joined by a companion on stochastic geometry, gives a good feeling for the exciting work that has gone on there. It originated in the idea of a memorial to Rollo Davidson, a brilliant mathematician whose tragic death in a mountaineering accident at the age of twenty-five deprived the subject of one of its most promising workers, and his friends of a delightful companion. Among the articles printed is posthumous work of Davidson, reconstructed from the papers which he left.

Although in one sense the book is historical, it is not backward-looking; the lines of research described are still very active, and there are many unsolved problems which pose a challenge

for the future. Nor, despite the fact that a dozen authors have contributed, does the book exhibit the miscellaneous character common in such collections; the influence of the warehouse, and of its director, runs as a unifying thread through the various papers. To Professor Kendall, and to his colleague Dr Harding, one owes a debt of gratitude for producing a book which will be of great interest not only to the specialist, but to the mathematician or statistician who wants to know what is going on in this field.

J. F. C. KINGMAN

Atmosphere and climate

Synoptic Climatology. By R. G. Barry and A. H. Perry. Pp. xvi + 555. (Methuen: London, October 1973.) £9.50.

MANY readers will have only a hazy idea of what is meant by synoptic climatology; indeed different authors have different ideas on the subject but in this volume the basic aim is to relate local or regional climates to a meaningful frame of reference—the atmospheric circulation. The authors recognise two stages in any synoptic climatological study, the determination of categories of atmospheric circulation type and the assessment of weather elements in relation to these types. Such studies cover a very broad field including long range weather forecasting on time scales from a week or so ahead to a decade or more.

The book is sensibly laid out with main sections covering history, data and analysis, synoptic climatological analysis, statistics, applications and future prospects. One of the best features is the bibliography of some eighty pages which covers much of the useful foreign literature as well as most of the modern work of the British Meteorological Office and includes works published up to 1973. I could detect no errors in the bibliography.

The volume is intended for students of advanced climatology at senior undergraduate or graduate level and for students of environmental problems. The authors have not, however, differentiated between the wheat and the chaff; they refer to important and unimportant aspects of the subject without a clear indication of which are the most essential. In chapter 2, for example, there is a good deal of obsolescent information dating from before 1950 about the frequency of cyclones and anticyclones and the tracks of depressions and only a few pages are devoted to discussion of the value of satellite pictures in synoptic climatological analysis.

Chapter 3 gives on the other hand a very good account of the various classification systems which have been devised to describe day to day weather on both regional and hemispheric scales.

Not only are the more well known classifications such as those of Lamb and the German Grosswetterlagen fully discussed but the various Russian methods are explained in a lucid manner which is not easy to do from translations of the originals which I have studied. Lesser known classification systems, such as those based on both surface and upper air conditions, for Italy by Gazzalo and for Switzerland by Schuepp, are welcome inclusions.

The chapter on statistical methods is rather patchy; the more elementary side is adequately covered but the chapter tries to cover too much ground which would be better dealt with in a suitable text book.

Since synoptic climatology is essentially a practical subject the devotion of 150 pages to applications is very reasonable; in fact this chapter could easily have been made longer. The best use has not, however, been made of the available space: there is far too much discussion about "singularities" and methods of defining natural seasons (curiously without reference to the Russian six-season system) and very little on important applications such as animal diseases, (only one reference to the extensive work of L. P. Smith) and human biometeorology.

The section on long-range forecasting gives some indication of the complex methods presently used, but does not give any clear idea of the relative importance of the different ideas. There is a fairly good section outlining the possibilities of climatic forecasting on the time scale of decades or longer and there is good appreciation of the importance of sea temperature anomalies in determining the various atmospheric modes.

The final chapter, though short, is an adequate summing up and indicates that there is a promising future for the subject in many fields. The book should certainly go a long way towards meeting university teaching needs and will be read with interest by many meteorologists. It is a pity that some of the figures are not clear, due usually to coastlines not being adequately distinguished from other lines but occasionally also to inadequate explanation; there are also a few typescript errors. Nevertheless the book is a welcome addition to the scientific literature.

R. A. S. RATCLIFFE

Erratum

In the review of the book *Immunopotential* (Ciba Foundation Symposium 18) (242, 322; 1974), the third paragraph should have started: "It seems likely that the agents useful in cancer act on the immune system" instead of "It seems unlikely . . ." as printed.



WILEY INTERSCIENCE



PHYSICAL GEOLOGY,

by Richard Foster Flint and Brian J. Skinner, *both of Yale University.*

Replaces Physical Geology by Longwell, Flint and Sanders; Reflects the revolution in earth science caused by the theory of plate tectonics, increasing awareness of the effect of man on environments and investigations of the Moon, Mars and members of the Solar System. In describing Earth's cyclical, dynamic processes, the concept of the steady-state condition (and its importance for living things) is repeatedly emphasized.

February 1974 510 pages £7.30

RADIANT HEAT TRANSFER IN A CLOUDY ATMOSPHERE,

by E.M. Feigel'son, *Institute of the Physics of the Atmosphere, USSR*

The monograph deals with the relationship between radiation and clouds and the introduction of radiant heat fluxes into problems of the dynamics of the atmosphere and cloud physics.

January 1974 196 pages £8.60
Published by Israel Program for Scientific Translations Ltd., and distributed by John Wiley and Sons, Ltd.

BRAIN CONTROL: A Critical Examination of Brain Stimulation and Psychosurgery,

by Elliot S. Valenstein, *Department of Psychology, University of Michigan.*

This new book by the well-known neuroscientist Elliot S. Valenstein traces the fascinating story of man's attempt to control his own brain. By extensively covering a wide range of topics, including the modification of behaviour and emotions in animals and man by electrical and chemical brain stimulation, psychosurgery, electroconvulsive shock treatment, prosthetic devices for the blind and the deaf, biological approaches to controlling aggression in children and adults, controlling pain and many other topics, the author has successfully succeeded in presenting an extremely scholarly and balanced text of the major attempts to modify the brain through physical interventions.

January 1974 430 pages £6.25

TRANSPORT PHENOMENA AND LIVING SYSTEMS:

Biomedical Aspects of Momentum and Mass Transport,

by E.N. Lightfoot, *University of Wisconsin.*

Provides a unified view of both biological flow and mass transport processes from a quantitative viewpoint. Concentrates on medically orientated aspects and progresses from flow to mass transfer and from defining equations to successively more complex applications.

February 1974 508 pages £12.50

AN INTRODUCTION TO MEDICAL STATISTICS,

edited by H.O. Lancaster, *University of Sydney.*

An introduction to medical statistics as applied to the scientific, rather than technological or administrative, study of medicine. Gives precise definitions, explains terms and interprets actual data from census enquiries, clinical trials and surveys and medical literature. Emphasizes understanding data on population, fertility and mortality. (Wiley Series in Probability and Mathematical Statistics: Applied Section).

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THE TOTAL SYNTHESIS OF NATURAL PRODUCTS, Volume 2,

edited by John ApSimon, *Carleton University, Canada.*

Describes some terpenoid and steroid syntheses, areas that have been the most studied from a synthetic viewpoint. Volume 1 deals with the total synthesis of a wide variety of natural products.

November 1973 766 pages £13.40

MICROPROBE ANALYSIS,

edited by Christian A. Anderson, *Hasler Research Center, Applied Research Laboratories, U.S.A.*

Studies the analysis and characterization of the microstructures of heterogeneous materials using microprobe instrumentation. Characterization is by means of morphology, chemistry and crystallography and the three main classes of instrumentation are the electron, laser and ion microprobes. Special advantages and area of application of each of the techniques and instruments are delineated.

September 1973 586 pages £13.40

ANHYDROBIOSIS,

edited by John H. Crowe, *University of California, Davis,* and James S. Clegg, *University of Miami.*

Examines the Nature of Life in a dehydrated state—the role of water in cells and how organisms adapt to survive. Editors' commentaries and author and subject indexes are included. (Benchmark Papers in Biological Concepts).

November 1973 492 pages £12.85

Published by Dowden, Hutchinson and Ross, Inc., and distributed by John Wiley and Sons Ltd.

MICROBIAL PHOTOSYNTHESIS,

edited by June Lascelles, *University of California.*

Gathers much of the available literature about microbial photosynthesis and offers an introduction to the possible methods of obtaining microorganisms with unique and unusual properties. Editor's commentaries and author and subject indexes are included. (Benchmark Papers in Microbiology).

January 1974 416 pages £11.80

Published by Dowden, Hutchinson and Ross Inc., and distributed by John Wiley and Sons Ltd.

ANIMAL COLONIES: Their Development and Function Through Time,

edited by Richard S. Boardman and Alan H. Cheetham, *both of the National Museum of Natural History, Smithsonian Institution,* and William A. Oliver, Jr., *US Geological Survey.*

Examines the fundamental nature of metazoan colonies, reviews much of the existing literature and provides new morphologic data from both fossil and modern animals. Papers on sponges, coelenterates, bryozoans and graptolites are included.

December 1973 618 pages £18.75

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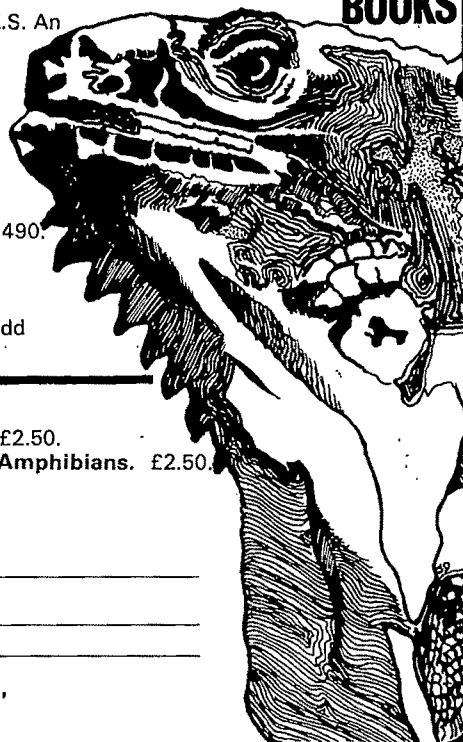
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matters arising

Haem deficiency and chain synthesis

SIR—The results reported by Giglioni *et al.*¹ on the differential effect of haemin on α and β chain synthesis are interesting. The authors conclude that only α -chain synthesis is stimulated by haemin. This contrasts with the conclusions of others^{2,3} that in a cell-free

that the selective effect of haemin acted at the level of translation, supports our earlier hypothesis explaining the relative depression of α chains. In haem-free cell-free systems, globin synthesis is inhibited after several minutes. This has been shown to be due to a soluble factor which inhibits the initiation of globin chains⁸⁻¹⁰. As α chains are normally initiated at a

⁹ Gross, M., and Rabinovitz, M., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1565 (1972).

¹⁰ Hunt, T., Vanderhoff, G. A., and London, I. M., *J. molec. Biol.*, **66**, 471 (1972).

¹¹ Clegg, J. B., Weatherall, D. J., and Euson, C. E., *Biochem. biophys. Acta*, **247**, 109 (1971).

Controlled use of nucleolus organisers

SIR—Macgregor has proposed that only some of the nucleolus organisers present act as the templates for transcription of rRNA in somatic cells, for the amplification of ribosomal genes (rDNA) in oocytes, and for repeated replication of rDNA in polytene chromosomes¹. This hypothesis evades the usually accepted requirement for a compensatory increased use of one organiser if the other one is defective or missing. There must, however, be regulatory devices to limit such usage, for example, a quantitative control of transcription, which do involve compensatory changes, and so the hypothesis provides no genuine economy of assumptions.

As in many other eukaryotes, a large proportion of diploid cells in *Xenopus* tadpoles have two nucleoli, both of which are engaged in RNA synthesis simultaneously^{2,3}. This is the evidence, overlooked by Macgregor, that both organisers are normally transcribed. As tadpoles which have only one organiser and form one nucleolus in each cell still achieve a normal rate of rRNA synthesis, it is hard to avoid the conclusion that the rate of transcription of the solitary organiser has doubled. Even fragments of organisers are active in heterozygotes, where they form miniature nucleoli⁴. Macgregor's hypothesis concerning transcription has thus a very limited application, being identical to the concept of latent organisers and known only in some interspecific hybrids. This allelic repression of one organiser has long been suspected to indicate a control system in plant hybrids⁵, but its molecular aspects are better demonstrated in mammalian cell hybrids⁶ and *Xenopus* hybrids⁷. These examples all provide evidence of compensatory changes occurring under a transcriptional control which does not discriminate between identical organisers nor apparently reduce their activity much below the normal rate,

TABLE 1 Relative rates of α and β chain synthesis by reticulocytes of patients suffering from haem deficiency disorders

Condition	No.	Mean $\alpha:\beta$	Effect of exogenous haemin
Congenital sideroblastic anaemia	3	0.5-0.8	0.85-0.90
Acquired sideroblastic anaemia	6	0.7-0.81	0.90-0.98
Lead poisoning	4	0.4-0.7	0.8-0.91*
Lead <i>in vitro</i> (1.5 $\mu\text{g ml}^{-1}$)	15	0.75-0.8	0.95-1.05
Iron deficiency	6	0.82-0.94	—

The α and β chains were isolated from globin prepared from unpurified whole cell lysates after a 60 min incubation with ³H-leucine.

* Observations on the reticulocytes of two patients.

system the stimulatory effect of haemin on protein synthesis is non-specific but is consistent with work, previously reported from this laboratory⁴⁻⁶, on the relative rates of synthesis of α and β chains in reticulocytes of patients who were haem deficient.

In 1971, reticulocytes of patients with congenital and acquired sideroblastic anaemia were found to show a low $\alpha:\beta$ ratio⁴. Later a similar depression in $\alpha:\beta$ ratio was found in children with acute lead poisoning⁵; an effect which could be produced by incubating normal reticulocytes in the presence of lead⁶. In all three situations the $\alpha:\beta$ ratio reverted to unity on addition of exogenous haemin. The results from all these four studies are summarised in Table I. Although these results contrast with observations of Tavill *et al.*⁷ using rabbit reticulocytes, we conclude that in human reticulocytes which are haem deficient, synthesis of α chains is depressed more than β chain synthesis. Thus, haem must have a differential effect upon the synthesis of these two chains⁴.

The conclusion of Giglioni *et al.*¹

slower rate than β chains¹¹, their initiation might be more sensitive to the action of the inhibitor.

Yours faithfully,

J. M. WHITE

A. V. HOFFBRAND

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¹ Giglioni, B., Gianni, A. M., Comi, P., Ottolenghi, S., and Rungger, D., *Nature new Biol.*, **246**, 99 (1973).

² Benzard, Y., Rodvieu, R., and London, I. M., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1022 (1973).

³ Mathews, M. B., Hunt, T., and Brayley, A., *Nature new Biol.*, **243**, 230 (1973).

⁴ White, J. M., Brain, M. C., and Ali, M. A. M., *Br. J. Haemat.*, **20**, 263 (1971).

⁵ White, J. M., and Harvey, D., *Nature*, **263**, 71 (1972).

⁶ White, J. M., and Piddington, S. K., *Clin. Sci.*, **44**, 20 (1973).

⁷ Tavill, A. G., Grayzel, A. I., London, I. M., Williams, M. K., and Vanderhoff, G. A., *J. biol. Chem.*, **243**, 4987 (1968).

⁸ Maxwell, C. R., and Rabinovitz, M., *Biochem. biophys. Res. Commun.*, **35**, 79 (1969).

as the presence of extra organisers in polyploid and aneuploid plants results in extra nucleoli and a proportionate increase in RNA^{8,9}.

Many of these examples can be repeated in terms of amplification, so it is only reasonable to suppose amplification is regulated in a similar manner to that outlined above for transcription. Macgregor¹ argues to the contrary, but lacks evidence; his observations on polyploid oocytes are ambiguous on this point and I doubt if his results on spermatocytes are even pertinent.

Similarly, when applied to polytene chromosomes, Macgregor's hypothesis is only a possible but unnecessary complication to the control mechanism envisaged by Spear and Gall¹⁰.

H. WALLACE

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University of Birmingham,
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¹ Macgregor, H. C., *Nature*, **246**, 81-82 (1973).

² Wallace, H., *J. Morphol.*, **112**, 261-278 (1963).

³ Wallace, H., *Nat. Cancer Monogr.*, **23**, 425-430 (1966).

⁴ Miller, L., and Gurdon, J. B., *Nature*, **227**, 1108-1110 (1970).

⁵ Wallace, H., and Langridge, W. H. R., *Heredity*, **27**, 1-13 (1971).

⁶ Bramwell, M. E., and Handmaker, S. D., *Biochim. biophys. Acta*, **232**, 580-583 (1971).

⁷ Honjo, T., and Reeder, R. H., *J. molec. Biol.*, **80**, 217-228 (1973).

⁸ Lin, M., *Chromosoma (Berl.)*, **7**, 340-370 (1955).

⁹ Longwell, A. R. C., and Svihla, G., *Expl. Cell Res.*, **20**, 294-312 (1960).

¹⁰ Spear, B. B., and Gall, J. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1359-1363 (1973).

obituary

V. C. A. Ferraro

V. C. A. FERRARO, who died on January 3, 1974, had been Professor of Applied Mathematics at Queen Mary College, University of London, since 1952; he expected to retire from his chair later this year. He had earlier (1947-52) been professor at the University of Exeter.

When he came to Queen Mary College the Department of Mathematics had six members; there are now three separate departments of Pure Mathematics, Applied Mathematics, Computer Science and Statistics, with a total of thirty-five lecturing staff. Professor Ferraro directed this expansion with energy and foresight, encouraging the growth of algebra, magnetohydrodynamics and astrophysics, the theory of computation and statistics.

He was best known for his share in the Chapman-Ferraro theory of magnetic storms, and for his discovery of the law of isorotation in cosmic masses. The Chapman-Ferraro theory was first advanced in 1930, when Ferraro was a

research student, and he returned to develop aspects of it over many years. The theory ascribed magnetic storms to streams emitted from the Sun into otherwise empty space, and the recognition that the solar wind flows all the time has meant that considerable modifications have later proved necessary. Moreover, the theory was a theory of the initial phase of a storm only; the type of ring current required to explain the main phase was not identified until much later. The essentially valuable new idea introduced in the theory was in its recognition that a solar stream must be regarded not as a collection of individual particles, but more nearly as a perfectly conducting fluid, able to modify the geomagnetic field by pushing the field-lines in front of it. The theory also introduced the idea of a magnetospheric cavity, though a temporary one.

The law of isorotation (1937), later much used in the theory of star formation, asserts that in a cosmic rotating mass of ionised gas penetrated by a magnetic field the angular velocity rapidly tends to become constant along

a magnetic field line. This result, like those of the theory of magnetic storms, seems today an almost obvious illustration of the principles of magnetohydrodynamics (MHD). But these principles were not understood when Ferraro made his discovery; he can fairly be claimed to be one of the pioneers of MHD.

In the postwar years Ferraro did much to encourage research in MHD, by his own example, by organising regular seminars and by the writing of a book on the subject with Plumpton (1961). He also is known for his book on Electromagnetic Theory (1953). He was a person of continuous activity; this activity was a little circumscribed after a heart attack in 1965, but he was publishing papers on the solar wind and related subjects up to the end.

But he will be remembered most in the College for his old world charm and personal courtesy, which is best conveyed in the words of one of Queen Mary College's cockney waitresses "He was a real gentleman, he was".

He leaves a widow and one son.

Announcements

Awards

The Trustees of the Lady Tata Memorial Trust invite applications for Fellowships and Scholarships for research on leukaemia, in the Academic Year beginning October 1, 1974.

The prizewinners of the Feldberg Foundation awards for 1974 are Professor G. R. Brindley FRS, Honorary Director of the MRS Neurological Prostheses Unit, at the Institute of Psychiatry,

and Professor P. Karlson, Physiologisch-Chemisches Institut der Universität, Lahnberge.

Errata

In the article 'Effect of Muscle Stretching on Tension Development and Mechanical Threshold during Contractures' by H. Gonzalez-Serratos, R. Valle and A. Cillero (*Nature new Biol.*, **246**, 221; 1973) paragraph 10, line 2 should read 'depolarisations produced with solutions containing 80 to 190 nM K⁺...'. Reference 2 should read volume 117 not 177.

In the article 'The Fluorescence of Quinacrine Mustard with Nucleic Acids' by Ritva-Kajsa Selander and Albert de la Chapelle (*Nature new Biol.*, **245**, 240; 1973) the labelling to the ordinate of Fig. 4 should read 'Fluorescence units at 514 nm' not 'Fluorescence at 514 nm (F)'. In Table 1 the heading of columns 2 and 3 should read 'Fluorescence units at 514 nm' not 'Fluorescence (F) at 514 nm'. The last sentence in the legend to Table 1 beginning 'Fluorescence was expressed...' should be deleted. The name of the first author should be Ritva-Kajsa Selander.

International Meetings

March 7, **Wild Oats, Economics, Biology and Herbicides** (D. T. Sagers, Fisons Agrochemical Division, Chesterford Park Research Station, Great Chesterford, Essex) (Venue changed from Shell Centre to British Museum (Natural History))

March 11-14, **Fast Reactor Power Stations** (Mr P. McLaren, Technical Secretary, c/o Fast Reactor Training Centre, Dere, Thurso, Caithness, Scotland)

March 22-25, **Practical Metallic Composites** (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20 9LW)

March 23-April 1, **East African Community Symposium on Aquatic Resources and Central Africa** (Dr J. Okedi, Director E.A.F.S.R.O., P.O. Box 343 JINJA, Uganda)

March 25-27, **Aslib Coordinate Indexing Group Conference** (Miss Verina Horsnell, Polytechnic of North London, 207-225 Essex Road, London N1 3PN)

March 25-27, **Moving Boundary Problems in Heat Flow and Diffusion** (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY)

March 25-29, **Petrophysics: The Physics and Chemistry of Minerals and Rocks** (W. F. Mavor, Administrative Assistant, School of Physics, The University, Newcastle upon Tyne, NE1 7RU)

March 26-28, **Nucleation, Folding and Interactions of Biopolymers** (Dr R. H. Pain, Department of Biochemistry, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE1 7RU)

March 26-27, **The Conservation of Materials** (Mr C. J. A. Preuveneers, Conference Secretary, Education Centre, B. 455 AERE Harwell, Didcot Berkshire OX11 0QJ)

March 26-28, **6th Cranfield Fluidics Conference** (Organising Secretary, 6th C.F.C., BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ)

March 27, **Corrosion in the Food Industry** (Mr K. Farrow, Fulmar Research Institute, Stoke Poges, Buckinghamshire) (Altered Date)

March 27-28, **International Technico-Economical Symposium** (i.b./c.c. Administration, Nieuwelaan 65, B-1820 Strombeek, Belgium)

March 27-28, **Rubber and Elasticity Symposium** (The Registrar, University of Manchester Institute of Science and Technology, P.O. Box 88 Manchester, M60 1QD)

March 27-28, **Speckle Phenomena and their Applications** (Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 8QX)

March 27-29, **Nuclear Structure and High Energy Physics** (Dr P. J. Negus, Department of Natural Philosophy, The University, Glasgow G12 8QQ)

March 27-29, **Minicomputer Interfacing** (Dr Yakup Paker, Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS)

March 28-29, **Biogenesis of Chloroplasts and Mitochondria** (Dr B. M. Richards, Searle Research Laboratories, Lane End Road, High Wycombe, Buckinghamshire)

March 28-29, **Recent Developments in the Petroleum Industry** (C. H. Maynard, Assistant General Secretary, Zoological Society of London, Regents Park, London N.W.1)

March 29, **The Genetical Society Brighton Meeting** (M. A. Ferguson-Smith, Institute of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS)

March 31-April 5, **Electronics in Medicine** (Centre for Extension Studies,

University of Technology, Loughborough, Leicester LE11 3TU)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Freshwater Biological Association. *Scientific Publication No. 28: A Key to the Adults of the British Trichoptera*. By Dr T. T. Macan. Illustrated by C. Joan Worthington. Pp. 151+5 plates. (Ambleside, Westmorland: Freshwater Biological Association, The Ferry House, Far Sawrey, 1973.) £1.25.

Ministry of Agriculture, Fisheries and Food. *Pest Infestation Control: Combined Report of the Director of the Pest Infestation Control Laboratory, 1968-70*. Pp. viii+205+12 plates. (London: HMSO, 1973.) £1.50 net.

Bulletin of the British Museum (Natural History). Entomology. Vol. 28, No. 6: *The Higher Classification of the Lycenidae (Lepidoptera)—A Tentative Arrangement*. By J. N. Elliot. Pp. 371-505+6 plates. £6.90. Zoology. Vol. 25, No. 8: *Relationships of the Palaeartic Lizards Assigned to the Genera *Lacerta*, *Algyroides* and *Psammis** (Reptilia: Lacertidae). By E. N. Arnold. Pp. 289-366. £3.35. (London: British Museum (Natural History), 1973.)

1974 Calendar—British Birds by British Artists. (London: British Museum (Natural History), 1973.) 75p.

Other Countries

American Geographical Society. *Antarctic Map Folio Series. Folio 16: Morphology of the Earth in the Antarctic and Subantarctic*. By Bruce C. Heezen, Marie Tharp and Charles R. Bentley. Pp. 16+8 plates. Folio 17: *Marine Sediments of the Southern Oceans*. By H. G. Goodell, R. Houtz, M. Bwing, D. Hayes, B. Naini, R. J. Echols, J. P. Kennett and J. G. Donahue. Pp. 18+9 plates. \$11. (New York: American Geographical Society, 1972 and 1973.)

Conservation for Survival: Ethiopia's Choice. By Leslie H. Brown. Pp. ii+96. (Addis Ababa: Haile Sellassie I University, 1973.) £1.20; \$3.

Fisheries Research Board of Canada. *Technical Report No. 411: Variation in the Food of American Plaice (*Hippoglossoides platessoides*) with Fish Length and Locality in the Scotian Shelf and Gulf of St. Lawrence*. By J. S. Scott. Pp. 15. (St. Andrews, NB: Fisheries Research Board of Canada; Biological Station, 1973.)

Australian Journal of Zoology. Supplementary Series No. 21: *Studies on Australian Muscoidae (Diptera). IV: A Revision of the Sub-Families Musconae and Stomoxyninae*. By A. C. Pont. Pp. 129-296. (East Melbourne: Editorial and Publications Section, CSIRO, 372 Albert Street, 1973.)

United States Department of the Interior: Geological Survey. *Professional Paper 529-K: Atlantic Continental Shelf and Slope of the United States—Sand-Size Fraction of Bottom Sediments, New Jersey to Nova Scotia*. By James V. A. Trumbull. Pp. iii+45. (Washington, DC: Government Printing Office, 1972.) 55 cents.

TABLE 1 Observations of λ -Sco

Date	Observation starting time (UT)			No. of integration periods	Source count	Background count
	h	min	s			
19/9/72	21	8	35	116	329	325
20/9/72	9	58	30	164	557	574
20/9/72	21	19	3	129	396	412
21/9/72	9	11	18	152	478	493
21/9/72	21	16	32	140	397	407
27/4/73	16	6	34	100	273	323
30/4/73	18	52	3	63	185	213
1/4/73	8	3	36	172	598	574
1/4/73	19	40	0	59	217	193
2/4/73	7	17	50	140	478	461
Totals	109 h			1,235	3,908	3,975

One integration period is 62.7 s long and the time between the start of successive integration periods is 86.5 s.

Table 1 of the article "Upper limit to the flux of soft X rays from λ -Sco" by K. T. Strong, M. W. Colley and J. L. Culhane which appears on page 34 of this issue. Due to production difficulties this table was unfortunately omitted from page 34.

nature

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No-one wins

WHAT did the general election in Britain mean—and what does it portend? British readers, worn out with journalistic analyses, will have to bear with us for a week for the sake of our overseas readers.

Hardly anyone in the political world can be very pleased by the present indecision. Mr Wilson heads a Labour Party whose number of seats falls short of the 318 needed for an absolute majority. Mr Heath, having gone to the country early to seek a strong mandate to deal with threats to his counter-inflation policy, found the country unresponsive to him. Mr Thorpe, having done wonders in making a Liberal revival look credible and worth voting for, gets 20% of the popular vote and only 2% of the seats in Parliament.

The campaign has been surprisingly moderate and serious in character. The central issue on which Mr Heath called the election was the deadlock between the government and the miners' union over pay increases, and it is clear that Mr Heath originally hoped that the country would see him as a moderate, fighting off union extremism. His motif has been 'firm but fair', repeated so often and so similar to the motif 'frei aber froh' of Brahms' Third Symphony that one was surprised Mr Heath, a cultured man, did not use the opening of that symphony to herald his electioneering appearances. Words become codewords in elections and 'firm' clearly was meant to signify 'tough on the unions' so here was an opportunity for a bitter, divisive election reviving all the class conflicts that periodically wrack the country. That this did not happen appears to have been due to two factors: the two major parties perceived that a doctrinaire battle would have allowed the Liberals to take the middle ground where the real majority always lies, and Labour was able to broaden electoral issues to the whole economic scene. Neither major party has been very explicit about what it would do for the miners, but it could be inferred that whatever the result, miners would get substantial raises, through some quasi-judicial process from Mr Heath or through some great emotional gesture from Mr Wilson.

What do the results portend?

The parliamentary system is about as close to deadlock as possible. Suddenly the much vaunted British system for maintaining two party democracy has creaked, for no leader can now ignore the Liberals and other parties which have led a shadowy existence for so many years. Liberals and Nationalists are at least relatively attractive partners for a coalition, less so the eleven Protestant Ulstermen dedicated to bringing down the tentative attempts at cooperative democracy in Northern Ireland.

Unfortunately, the national skill for compromise is poorly exercised in putting together coalitions. Labour

in particular still has bitter memories of the thirties when a coalition nearly annihilated the party. Both major parties have been hurt by the swing to the Liberals, but Mr Wilson can soldier on for maybe a year or more with the grudging support of the Liberals and Nationalists, and then turn to the electorate with a good chance of gaining an absolute majority. This Mr Heath must have perceived for he initially refused to resign and has attempted to put together a government of his own by courting the Liberals.

Much, however, must now change politically. The Liberals could well force electoral reform. The Nationalists could well force devolution of power on many issues to regional parliaments. Most important, if the grip of the two major parties, both so clearly identified with class distinction, can be loosened, it is possible that in the next fifty years Britain, still grossly class ridden, can develop a more equitable society. Neither party is able to achieve that at present.

100 years ago



IN a recent number we intimated that the Perthshire Society of Natural Science had interrogated the Parliamentary candidates for the county and city of Perth as to their opinions on the questions of State help to Science, a responsible Minister of Education, and the promotion of Scientific Exploring expeditions.

Answers—favourable, we are glad to say—were returned at the time only by the two Conservative candidates, one of whom, Sir W. Stirling Maxwell, is now M.P. for Perthshire. We are now glad to give place to the somewhat tardy reply, addressed to the secretary, of the Hon. Arthur Kinnaird, Member for the City of Perth:—"I, Pall Mall East, 18th Feb. 1874.—Dear Sir,—I was surprised to find copied into a London paper from a Scotch journal the questions put in your letter of the 29th January last, with the statement that they had not been answered by me. The fact of my being, as I believe I am, one of the patrons of the Perthshire Society of Natural Science should have been, it appears to me, a sufficient guarantee of my approval of the objects of your institution; and my active co-operation with Capt. Wells in his efforts during the last session of Parliament to obtain the sanction of Government to a proposed grant for the furtherance of Arctic exploration, further approves my appreciation of the objects you advocate, in my willingness to support State expenditure for well-devised schemes of scientific research and educational purposes.—Yours truly, A. Kinnaird."

From *Nature*, 9, 350, March 12, 1874.

Decisions about the European computer industry

In deciding whether to approve more funds for Compagnie Internationale pour l'Informatique (CII), the French member of the European computer grouping Unidata, the French government is having to weigh up the long term prospects of, for example, an alliance with an American company. Professor A. S. Douglas, Professor of Computational Methods at the London School of Economics and Past President of the British Computer Society examines the underlying issues.

EUROPEAN cooperation, as has been recently demonstrated in Washington, is often difficult to achieve. Nowhere had this proved more true than in computing, and the latest report of the problems of CII is only one further manifestation of the phenomenon.

Case for indigenous industry

There are some very obvious reasons why an indigenous computer industry is desirable in any country. Industry, commerce and government itself are all coming increasingly to depend on the efficiency and speed of computers in handling clerical tasks, plant control and planning studies. The industry is already the third largest, in terms of turnover, after oil and aircraft. At least one company in the industry, IBM, is highly profitable. Thus each government, nervous of the effect which foreign control of this key resource might have on its own efficiency and that of local industry, seeks to protect itself and its 'charges' as best it can, and local interests seek to participate in the profits which should flow from a new and growing industry.

Since the computer industry has its roots, in the main, in the United States, the general fear of external interference may be made more or less pointed by the political climate existing between any country and the United States. The more concern over political implications, the more urgency is there to avoid undue dependence on technology from the United States; the stronger the local interests in the industry, the more concern there is to build up local industry under some form of protective umbrella which will hamper United States activity locally.

The arguments which have led many governments, including those of Britain, France, West Germany and Japan, to subsidise and protect their local interests against the supposed 'threat', are, of course, valid concerning neighbouring governments which may be thought to hold an important card in the industry. Thus cooperation is apt to be approached in a spirit of suspicion. Only if the external threat to a group is deemed strong enough, or if there is an obvious pay-off for all, is it likely that the suspicions will be overcome and a real measure of cooperation take place.

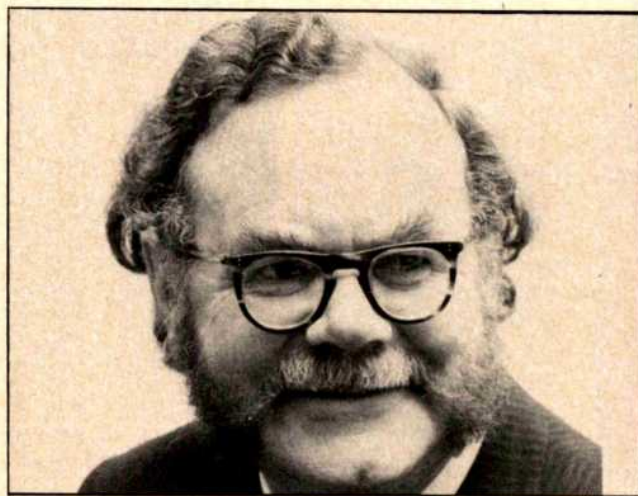
Arguments for cooperation

Setting aside the strength (or otherwise) of this 'threat' from the United States—on which opinions will clearly differ—and the rather more general sentimental arguments in favour of cooperation within Europe, the primary arguments for cooperation are commercial. Computers, even minicomputers, are relatively complicated machines, both to

build and to maintain. Good design makes for reliability and ease of maintenance but such design only results from the incorporation of experience. Experience is gained from use, and is the more quickly learned the more machines are sold and used. Thus quantity production leads not only to reduced unit costs, as with all products, but also to a more sharply convergent 'learning curve' towards the lower unit costs themselves.

There is, then, a double prize for achieving quantity production, and this can only be done in a large market. Europe is a fast-growing computer market and will, no doubt, in time become similar in size to that in the United States. A truly European marketing strategy, if pursued without political hampering, ought to be sound commercially. A sensible selection of points of attack on the dominance of the United States ought to succeed, whether or not governmental support is available, provided the companies concerned are at least as well managed as their competitors. All companies are, however, somewhat vulnerable in the course of their early developments and when a major investment in say, a new range of computers is being made, so perhaps there is a case for support. What is really needed is access to the whole European market on a basis of parity in each country and the price for this seems to be 'multinationalisation' of the firms concerned.

Because I believe that there is sound commercial sense in aiming to achieve a freer market situation, I would suppose that governments will press forward with collaboration—though obviously on the best terms each feels it can get for its own protégées. Probably, therefore, a solution will be found to the problems of CII and cooperation with Siemens and Philips will continue.



Professor A. S. Douglas

Britain

Meanwhile the British position requires careful consideration to see what is likely to be in the interest of British industry as a whole. Clearly ICL is an important part of the industry and its interests must be considered. But there are important sections in peripherals, minicomputer terminals, software and bureau operation, where government action can have equally important influences and where money is to be made both at home and abroad by good husbandry. So far, the Department of Trade and Industry has tended to pay very little attention to these areas and has concentrated its policy around the support of ICL alone. Recently there have been welcome signs of change, and it is to be hoped that a broader view will be taken in the future.

international news

Behaviour modification in the limelight

Colin Norman, Washington

IN the past few weeks, the topic of behaviour modification has been accorded considerable public attention and a good deal of newsprint in the United States. The sudden burst of publicity stems from two separate decisions by government agencies to withdraw support from potentially embarrassing projects, and the affair bears witness to the strong and growing public concern about research projects involving human experimentation.

The first government action to arouse interest was a decision by the US Bureau of Prisons to terminate a controversial project at a federal psychiatric prison at Springfield, Missouri. Called Start (an acronym for special treatment and rehabilitation training), the project involved rewarding good behaviour by granting so-called privileges to the inmates. The project was terminated, according to the Bureau of Prisons, because it was too costly, but the decision came after a group of inmates had filed suit in the federal courts, contending that Start violated their constitutional rights because it constitutes cruel and unusual punishment.

The second, and perhaps more important, episode was an abrupt decision by the Law Enforcement Assistance Administration (LEAA) to withdraw funds from projects involving "psycho-surgery, behaviour modification—including aversion therapy—and chemotherapy". According to LEAA Administrator Donald E. Santarelli, the decision was taken because the agency lacks the skills to monitor such research, and in any case, the projects are peripheral to LEAA's business of providing assistance to state and local law enforcement activities.

Those two incidents seem to indicate that the Nixon Administration has taken a critical look through its programmes involving human experimentation and has decided to weed out the more questionable among them. Unfortunately, however, that is not the

case and the two actions by themselves clear up none of the questions about the acceptability of behaviour modification, nor do they indicate any new policy throughout the Administration on human experimentation.

For one thing, the debate has so far been completely unencumbered by a definition of what constitutes behaviour modification, and for another, the two decisions were taken for bureaucratic, as opposed to ethical, reasons. But the actions will have beneficial consequences, and if nothing else, the public attention indicates that the concern over safeguards on human experimentation—a concern which owes much to the revelation in 1972 that a federally sponsored research project had denied treatment to a number of poor black men who were known to have syphilis—is making it much more difficult for questionable projects to carry on unnoticed.

As for the LEAA decision, Santarelli announced that his agency is getting out of the business of medical research and behaviour modification entirely, and that proposals for funding such research in future will simply be referred to the Department of Health, Education and Welfare. The agency, he said, is now conducting a search of the projects it has been funding, to see which fall into the forbidden category.

Given those statements, it may reasonably be asked what controls the agency has been applying to safeguard the rights of subjects in research projects it has been funding. The answer is pretty obvious from the fact that the agency does not even know what research projects it has been supporting, although its own guidelines dictate that each project involving medical research "should receive individual and prior approval by LEAA". Most LEAA money is provided in the form of block grants to state and local governments, and thus the agency is not always fully aware of every individual project receiving its support.

Without a definition of what constitutes behaviour modification, it is difficult to know exactly what LEAA intends to scrap, but some of its projects are clearly in jeopardy. It has been funding a project in Puerto Rico, for example, which involves the use of drugs in an attempt to see whether there is a link between criminality and brain damage. The agency has also

been supporting a project in Pennsylvania which is designed to test B. F. Skinner's theory of reward conditioning in the treatment of young offenders. Those projects will clearly lose their LEAA funds, but there is a whole grey area of projects that cannot easily be categorised, and whose future is therefore uncertain.

LEAA's decision is also likely to have hammered a nail into the coffin of a controversial proposal to establish a Center for the Study and Reduction of Violence at the University of California, Los Angeles. The centre which was proposed in 1972 and which was embraced by Governor Ronald Reagan in his State of the State message in



LEAA Administrator Donald E. Santarelli

January 1973, had applied for a \$750,000 block grant from LEAA, and for a similar amount from the State of California. LEAA had already consulted HEW on the matter, but it will now wash its hands of the proposal and the ultimate decision on whether to fund the centre will probably rest with HEW.

The centre has already caused a huge amount of controversy at UCLA and it has come to national attention largely through the work of a Washington-based public interest group called the Children's Defense Fund. Proposals for the centre, which have undergone many revisions since they were first put

forward in 1972, include investigations of the use of cyproterone acetate, a controversial so-called castration drug, on sex offenders; investigations designed to determine whether there is any correlation between abnormal electrical activity in the brain and violent behaviour; studies looking for correlation between criminal behaviour and the possession of XYY chromosomes; and biofeedback and aversion therapy for sex offenders. The centre's plans, which have been drawn up by its proposed director, Dr Louis Jolyon West, have been strongly criticised, by both public interest groups and by individuals who have examined them. Given the publicity that has already been attracted by the centre, it seems unlikely that it will be able to get through the network of review committees that HEW requires for research involving human subjects.

HEW, in fact, now has under consideration a proposal for strengthening its control of human experimentation (see *Nature*, 246, 239; 1973), and the Congress is considering a bill to strengthen the rules even further. Thus the LEAA decision will have the effect of shifting research into an agency where it will be reviewed rather critically on ethical and legal grounds.

As for the decision of the Bureau of Prisons to scrap the Start programme, that action is less far reaching, but nevertheless it is an important indicator that even the officials who operate the federal prison system, who have been heavily criticised by prisoners' rights organisations and other groups, are not unaware of the embarrassment potential of questionable human experimentation.

Last week, in fact, Mr Norman Carlson, Director of the Bureau of Prisons, and Dr Martin G. Groder, the Warden-designate of a medical research prison being built at Butner, North Carolina, were called before a committee of the House of Representatives to explain the bureau's behaviour modification studies. Both men denied that any aversion therapy is being carried out in federal prisons, and they agreed that the Start programme had some faults, chief of which is that the prisoners selected to take part had no choice in the matter, thereby violating one of the chief safeguards in any human experimentation: informed consent.

The committee was anxious to know about the plans for the Butner facility, and about the safeguards that the bureau will provide to ensure that research carried out there does not violate prisoners' rights. According to Groder, the prison, which will be capable of housing up to 340 prisoners, will be partly a medical treatment facility for prisoners with severe mental disorders, and partly a correctional research facility. As far as the

latter function is concerned, it will test various psychological and therapeutic methods of behaviour control, such as group therapy on the Synanon drug treatment model, and those that are found to be successful will be applied throughout the prison system.

Groder specifically stated that "none of the methods (of behaviour control) already preliminarily chosen or being considered favourably, involve the methods of modern-day torture known as aversive conditioning, specifically the misuse of drugs, electric shock or psychosurgery". That was greeted with favour by the committee, but the Congressmen were less impressed with the proposed methods for ensuring that prisoners taking part in the research programmes do so entirely of their own accord.

Groder made clear that those who will take part will have every opportunity to decline to take part, or opt out once the programme has started. But he also said that the prisoners selected to have a chance of taking part will all be within a few months of being considered for parole. At that stage of his incarceration, a prisoner is likely to do whatever he can to help assure his release—including taking part in a research project.

Nevertheless, the upsurge of public interest in safeguarding subjects of human experimentation should ensure that the Butner facility is kept under close and critical surveillance.

Vets make pay claim

John Hall

FIVE hundred veterinary surgeons employed by government departments in Britain have started a work to rule in support of a pay claim calculated to be in the region of 20%. The claim is vaguely defined and long delayed because the pay of veterinary officers is tied to that of scientists in government service, who are themselves waiting for action on a protracted and bitterly pursued claim. In hard cash terms, however, the vets are clear about their long term aims: an increase of £900 to £1,000 a year in the pay of grade one veterinary officers, currently earning £2,996 to £4,004.

Their industrial action threatened the government's brucellosis eradication programme and day-to-day essential work like the implementation of food orders and quarantine supervision. They gave a specific undertaking that they would not halt any work whose interruption would cause a health hazard,

either to animals or humans: the last time they threatened industrial action in similar terms, swine vesicular disease broke out the next day and their disruptive plan was called off.

Announcing the work to rule, the vets' union, the Institution of Professional Civil Servants (IPCS), painted a picture of a sadly declining profession, manned by middle-aged gents whose retirement will leave the service in a state of virtual collapse. Mr Cyril Cooper, Deputy General Secretary of the IPCS, said the service is operating on a manpower 30% below the required complement and that in the next eight or nine years something like half of the vets in government service would have reached retirement age, leaving the department in a "disastrous" situation.

Recruitment has almost come to a standstill because of the disparity between the salaries of vets employed by the state and those in private practice. In 1972, 300 graduates entered private practice, compared with eight entering government service, and of the 8,500 vets at work in Britain the state service accounts for a diminishing group of less than 5%. Although an arbitration tribunal in 1968 expressed doubts that a cash settlement would improve recruitment, and a more recent working party suggested that changes in the structure of the profession might offer a solution, the vets themselves have no doubt that the disastrous recruitment figures can only be reversed by a salary review on a grand scale.

They point out that since the beginning of 1971, the cost of living index has risen 28%, and wages generally have risen by 41%. Their own salaries have risen by between 19.5% and 26.5% depending on grading and so, in order to bring state vets back to their former position, a relativity increase of about 20% is needed. At present, salaries paid to the three grades of Government Veterinary Officer are: grade one, £2,995 to £4,004; grade two, £2,616 to £3,312; and Divisional Veterinary Officers, £4,240 to £5,912. They suggest that their future salaries should be compared with those of doctors, who can expect between £6,700 and £7,200 a year, and with dentists, who may be £600 below that level.

The Veterinary Service, they say, has operated for years on the good will of vets who were willing to cut corners and do work which they were not strictly required to do. But the goodwill is now in scarce supply, and since governments of different political complexion have shown themselves to be equally bad as employers, the vets thought they could only make their feelings known by applying pressure based on industrial action. They will do no overtime, give no lectures outside working

hours, take no work home, take time off in lieu of any weekend work they have to do, refuse to use their home telephones for official calls and stop doing jobs which ought properly to be done by an officer of a higher grade. Unless there's an outbreak of foot and mouth, that is.

Soviet breeder reactor accident

from our Soviet Correspondent

RECENT unofficial reports, apparently based on data from United States satellites, indicate a serious accident to the Soviet fast breeder reactor BN-350, which formed the heart of the new town of Shevchenko on the Mangyshlake Peninsular on the Caspian Sea.

The reactor was designed to power a desalination plant producing more than 100,000 cubic metres of fresh water a day and was intended to transform the desert shore of the Caspian "where even the camels look weary" into a modern, industrial model township with a population of 80,000, complete with parks, fountains and avenues of shady trees. The town was clearly intended to be a showpiece, and was accordingly named after the nineteenth century Ukrainian poet Taras Shevchenko, although the "drowsy waves, sky unwashed and dirty" and the "worthless sea" to which he was exiled was not in fact the Caspian but the Aral. Shevchenko was, however, a figure of world renown (even meriting a statue in Washington DC) and was considered a suitable eponymous hero for what was intended to become a showpiece of national and international importance.

Reports of the disaster pose some interesting problems when compared with the official publicity handouts. According to the Novosti Agency, the reactor, which was commissioned on September 27, 1972, was officially "launched" on July 16, 1973. Shortly afterwards an *Izvestia* correspondent visited the station and described in glowing terms the reactor with its control centre "like the bridge of an imaginary spacecraft". An influx of visitors was clearly expected—a special display showing a mockup of one of the fuel elements was exhibited in the entry hall. Even the *Izvestia* correspondent, however, noted the hazards of the "exotic coolant", molten sodium: but he stressed that special safety measures had been incorporated in the design.

The BN-350 was designed to operate on the three-circuit principle: in the first circuit the sodium flows directly round the fuel elements (steel "pencils" containing 6 mm pellets of uranium-235-

enriched uranium) and itself becomes radioactive, giving off heat through the steel heat exchanger walls to the molten sodium and the second circuit which is heated to 450° C. The sodium in the second circuit then heats the water in the third circuit in the usual way, producing a nominal 1400 tonnes of steam an hour. In all, the reactor has six such three-circuit loops. The total thermal power of the reactor is rated at 1 million kW (1GW) which can be divided between electricity generation and desalination as required. Initially, an optimal regime of 150,000 kW of electricity and 12,000 tonnes of desalinated water a day was envisaged.

The reports and rumours of damage suggest that something has failed in the cooling channels—though whether this refers to the first or second sodium circuit is not definite. Clearly, either could cause the "large fire" which the satellite data are said to indicate: damage to the first circuit, however, would be far more serious, involving a considerably greater radiation hazard.

The most curious feature is the date of the alleged disaster. That generally

mentioned is July 1973, shortly after the official "launching". But as late as August 21, 1973 the Novosti agency issued a three-page report on the BN-350, the "atomic heart" of the Mangyshlake Peninsular (a length of coverage normally given only to matters of major international importance). Either the satellite dating is incorrect, or the Novosti agency was particularly late in releasing information, or some attempt was made at a cover-up operation—a not unlikely contingency, in view of the prestige attached to developments at Shevchenko.

Chemists think about energy

IN Britain a group of top university chemists has been talking with scientists from Imperial Chemical Industries, British Petroleum and the Department of Energy in order to define long term prospects for evolving alternatives to fossil fuel supplies. The meeting followed an initiative by Professor R. Mason, Chairman of the Science Board of the Science Research Council (SRC), and among the academics brought in for the talks were Sir George Porter, Professor Sir Derek Barton and Professor Geoffrey Wilkinson. Similar discussions are likely to take place in other departments of the SRC, and a joint communique is expected in a month or so. In the meantime the Chemistry Study Group has issued the following statement:

"The Science Research Council has a broad responsibility for promoting research of 'timeliness and promise' which, together with its involvement in postgraduate education, can be expected to provide advances in fundamental scientific knowledge. However, within this general remit, the council is concerned to assess the balance of its funding pattern in relation to national needs or priorities. In common with many other scientific research funding agencies throughout the world, events of the past three months have caused it to take a new look at topics of research which may have a bearing on the energy situation.

A Chemistry Study Group of the Science Board (Secretary, Dr S. M. Mellows) has recently debated preliminary views of possible chemical innovation which might be applicable in the general context of energy supply. The Study Group accepted that any short term solutions to the problems in this area will most probably be based in industry, or, say, the Department of Energy rather than in the universities or polytechnics and, therefore, concentrated on longer term prospects such as the need for synthetic fuels in the year

Switched off in academe

A SUGGESTION that universities might lose their exemption from the working of the three-day week in Britain has been made in a memorandum issued to heads of departments and administrators at the University of Birmingham. Asking that the use of academic buildings in the evening and at night should be discouraged, the university's Estates and Buildings Officer, Mr J. H. Fathers, told members of staff that he was making the request as a result of an urgent message from the University Grants Committee (UGC).

"On Monday morning" the memo reads, "I received an urgent telephone call from the UGC telling me that the Department of Energy had contacted them to say that representations had been made about the excessive use of electricity in educational establishments. The department inferred that if this continued universities might be specifically placed under the regulations of the three day-week Statutory Order".

The UGC said it had no knowledge of an urgent telephone call and the Department of Energy denied making any inference that universities might be subject to the Statutory Order.

2000. Contributions to the Study Group's discussion from the wider chemical community would be welcomed.

The Study Group's first concern was to assess the possibilities for the fixation of atmospheric carbon dioxide. There was general agreement that emphasis on a biological photosynthesis would be justified, the photochemical synthesis, perhaps metal-catalysed, of organic molecules such as methanol or formic acid appearing to be a challenging and reasonable objective. Indeed much more knowledge generally of the photochemistry of related simple systems, such as the carbonate ion, is needed. The organometallic chemistry of carbon dioxide was discussed at some length and it was recognised that there had been little work directly related to this area; the Study Group's view might be summarised by saying that, although much would accrue in the general field of synthesis, it was not obvious at this time that homogeneous catalytic fixation of carbon dioxide was either technically feasible or economically viable. New developments of heterogeneous catalytic alloys could aid the separation and fixation of atmospheric carbon dioxide but an alternative technology based on molecular sieves appeared to be economically unattractive. It appeared that a single step conversion of methane to higher hydrocarbons or to higher alcohols would be of considerable interest to industry in the short term.

It was accepted that the further development of photovoltaic and photogalvanic cells should be of high priority and that the latter have potential for energy storage. The development of efficient fuel cells, similarly, is of importance but, in view of the heavy industrial investment in this area, SRC's interest should be restricted to well-defined new ideas such as alternatives to platinum electrodes. The low (about 80° C) temperature storage of hydrogen by both organic and inorganic systems emerged from the discussion as a promising area of enquiry while coordination of organic synthetic methods with solid state physics could provide new approaches to the development of the very elusive high temperature superconductor.

The Study Group emphasised that overdependence on nuclear energy for future needs, without heavy investment in research devoted to the utilisation of solar energy, could be hazardous. Of course, heavy investment in new research in these areas will require examination of the technological economics in relation to possible alternative methods. The Study Group will meet again to review any changes in the pattern of research activity and development which might originate from the chemical community as a whole."

Physicist, heal thyself

Michael Stone, East Berlin



Albert Einstein

THE mind boggles at the idea of an opera about somebody as unobtrusive and, generally, devoid of drama as Albert Einstein. The man acknowledged as this century's greatest scientist may have wrought a revolution in modern physics and astronomy, he may have shared responsibility for the development of nuclear research, but his own life was singularly free of anything approaching the sensational. His only personal experience of something nasty happened in 1933, when his books were among those burnt on Goebbels's orders and some Nazis wrecked his study in Berlin. Soon after, he emigrated to the United States where at Princeton University, he continued his studies. In August 1939 he wrote his famous letter to President Roosevelt which is said to have initiated the American nuclear research programme.

In East Germany, where "Einstein" has just had its world première at the Deutsche Staatsoper, mathematics are regarded as almost an Olympic discipline, with annual regional and national competitions, culminating in mathematical championships at different age levels throughout the Socialist countries. Fortunately for the rest of us Paul Dessau and his librettist, Karl Mickel, were more interested in the conscience of Albert Einstein and the success or failure of his endeavours as a pacifist and humanist. Dessau—for many years Bertolt Brecht's closest musical collaborator and friend—combined this subject with an ambition to revive the traditions of the popular musical theatre, from which have sprung such comic figures as the Shakespearean

fool, the harlequin, and Papageno. So he embedded the three acts showing up the relationship between politics and science—first in Nazi Germany, later in the United States—in a comic opera frame, with a prologue, two intermezzi and an epilogue.

Hans Wurst, a traditional comic character in Germany, makes a brief appearance to introduce the opera's basic idea: since science depends upon those in power, scientists ought to develop a social consciousness to make sure that their work serves humanity. In the first entracte, Hans Wurst, (which means as much as Jack Sausage) is thrown to a huge crocodile, symbolising Nazi Germany, but manages to save himself by the expedient of telling the beast a joke until outsize tears of laughter roll out of its eyes. The same trick does not seem to work during the second intermezzo—the crocodile now stands for the America of President Truman and Senator McCarthy—and he is gobbled up. In the end, a resurrected Hans Wurst balances delicately along the edge of an enormous razor, telling us how much he enjoys being alive.

In between such shenanigans we are treated to a semi-historical essay on the role of Einstein and other scientists under Hitler and in the US, the conclusion of which is that Einstein destroys his latest findings to prevent them being misused as, in his opinion, the discovery of nuclear fission had been perverted to blot out Hiroshima.

If this sounds like dry stuff, it isn't. Dessau is a fox who knows exactly how to garnish his score with musical titbits—here a melodious quote from Bach's Toccata, there a series of percussion effects—so that the ear craves for more. The singing is recitative rather than arioso, the orchestra relies more on the brass and the woodwinds, while the strings function mainly in parody; another pointer to Dessau's critical detachment towards the hero of his opera: Einstein was a lover of the violin.

In her production, Ruth Berghaus's scenic humour—she is Paul Dessau's wife and succeeded Helene Weigel as head of the Berliner Ensemble—well matches her husband's playfulness. In the sets of Andreas Reinhardt she provided Theo Adam (looking quite astonishingly like Einstein himself), Peter Schreier, and Reiner Süss (as two other physicists, one of whom makes his peace with the Nazis, while the other lands in gaol) with ever new stage arrangements, ensuring a pace and a high level of acting.

Reprinted with permission from *The Guardian*, February 26, 1974.

A silent summer in Washington State

Colin Norman, Washington

THE US Environmental Protection Agency (EPA) has given the go-ahead for what could be the most extensive use of DDT in the United States since the pesticide was banned in 1972. After much soul searching, EPA Administrator Russell E. Train announced last week that DDT can be used this summer to control an infestation of Douglas fir tussock moths, whose larvae have already munched their way through nearly a million acres of fir forests in the Pacific northwest. Although Train said that his decision was reached "reluctantly", he had little choice in the matter.

The moth, which is endemic to the forests of Washington State, Oregon and Idaho, suddenly began a population boom in 1970, and has been eating up an increasing area of foliage ever since. Such infestations usually end in the third year from natural causes, chief of which is a virus infection which spreads through the eggs in the winter and kills off the larvae when they hatch in the summer.

But this population boom failed to follow the three-year pattern, and so timber producers, farmers and the US Forest Service (part of the Department of Agriculture), petitioned the EPA to lift the ban on DDT in order to spray the larvae when they hatch in late May and early June.

The agency was forced to capitulate to demands for DDT for a variety of reasons. First, it refused to accept a similar petition last year on the grounds that the infestation would be wiped out by natural causes, without any help from pesticides. When several hundred acres of denuded trees starkly underlined the moth's refusal to follow past trends, the EPA was made to feel rather embarrassed. Another unfulfilled prophecy this year would be even more embarrassing and would add considerable impetus to attempts by a few Congressmen to strip some authority from the EPA.

Second, there are no alternative pesticides on the market which are known to be as effective as DDT in controlling the moth—a fact which Train last week blamed on the Forest Service because its research and testing programmes "have been almost totally inadequate—to the point of dereliction". And finally, the EPA was under court order to make a decision on DDT use by March 1, which is too early to tell whether there is indeed any chance of a virus infection killing off the pest this year.

The EPA was, of course, reluctant to allow use of DDT on such a large scale because it does not want to open the floodgates to pleas for special treatment for other pest infestations. Thus Train made clear last week that "my approval of this contingency request should in no way be viewed as a departure from the principles of EPA's June, 1972 order cancelling most uses of DDT. I remain personally convinced that the use of DDT represents a significant risk to ecological systems and that its use should be avoided wherever possible".

Train says that he has, in fact, extracted a promise from the Forest Service that DDT will only be used if necessary. In other words, if laboratory and field studies indicate that the population will be wiped out in some areas from virus infection, then the DDT canisters will remain unopened. The first indications of how the virus infection is progressing will come in the spring when egg masses collected last December are hatched in the laboratory.

If it turns out that DDT will be needed—and it can safely be assumed that the Forest Service will leave little to chance—then the EPA has laid down some fairly stringent rules. Spraying will begin when the eggs begin to hatch in late May, and end by the beginning of July. The pesticide will be applied by helicopter, but an unsprayed buffer strip must be maintained along streams and rivers, no spraying can take place in winds greater than 6 miles an hour and public warnings must be placed in all areas to be sprayed.

Apart from being somewhat embarrassed that their predictions of an imminent collapse in the tussock moth population failed to materialise last year, some EPA officials are also annoyed that DDT spraying has become necessary at all. One official pointed out last week that although the US Department of Agriculture itself banned use of DDT on forested areas in 1968, the department has failed to sponsor an adequate programme to develop alternative pesticides. It was only last summer, in fact, that large-scale field tests of alternatives were conducted, and even then no comparative tests with DDT were conducted. Consequently, EPA has no statistical evidence on the efficacy of DDT.

Train has therefore required, as a condition for approval of DDT use this year, that the Forest Service must undertake a "fully funded, comprehensive research effort on tussock moth control which will support registration of effective and environmentally acceptable alternatives to DDT next year".

Medical museum to move?

Fiona Selkirk

EXHIBITS from the Wellcome Trust's History of Medicine Museum may soon find a new and permanent home in the Science Museum. The Wellcome collection, assembled by Sir Henry Wellcome, contains many fine examples illustrating medical and social history, some early microscopes of excellent quality, fearsome yet interesting surgical instruments and about 200 pestles and mortars, in addition to Sir Henry's more personal collection of horse brasses and the like. The vast medical collection is very valuable and well worth exhibiting.

For many years the Wellcome Trust has been reorganising the museum and the library associated with it, and it has finally come up with a proposal which, with approval from the Department of Education and Science (DES), will mean the wholesale removal of the museum collection, but not the library, to the care of the Science Museum. (Specimens from the History of Medicine Museum can be seen in a current exhibition at the Science Museum, and in July more exhibits will be included in a display commemorating the bicentenary of the discovery of oxygen.)

Unfortunately, it seems that the Science Museum will have to do what the Wellcome Trust is forced to do at the moment—store between 85% and 90% of the collection—but if the proposal is accepted and all goes according to plan, by the end of 1976 there will be a gallery with 10,000 square feet of floor space, for a permanent exhibition of medical history.

At present, the students of medical history who use the facilities of the Wellcome museum and library have full access to the museum specimens in store. A spokesman for the Science Museum said that if all goes ahead, it is hoped to provide the same, and possibly even better, facilities—although, of course, without immediate access to the library.

The DES, which will have to approve the plans for financial reasons, has been kept informed but there have been no definite talks about the final proposal yet.

The Wellcome Trust awards grants for scientific, medical and clinical research, primarily in areas which do not receive large amounts of money from such grant-awarding bodies as the Medical and Science Research Councils. Of a total of nearly £2 million allocated in grants in 1972-73, about £350,000 (18%) went to history of medicine, but the trustees thought that no more

than £250,000 of that allocation could be spent on the museum and library. The target for 1973-74 is £365,000, of which about £90,000 will go in grants and to the units for the history of medicine at Oxford, Cambridge and University College London. It seems that the advisory panel feels that as the trustees wish to devote more funds in future to the promotion of study in the history of medicine the remaining £275,000 this year, and, possibly relatively less subsequently, will not be sufficient to finance the upkeep and development of both museum and library. The panel thinks it is important to develop the latter as a major source of information, not only about medical history but also about more modern medicine.

Museum collections have to be catalogued, maintained and well exhibited, all of which require time, effort and money. The trustees obviously believe that they cannot provide these and so justify the permanent loan of their collection to the Science Museum. There, they feel, the collection can have the care and attention it deserves.

This may be so, but the transfer of what was part of a charitable institution to the care of one funded by public money, regardless of the initial financial assistance proposed by the trust, must come under close DES scrutiny, especially when the time comes that the Science Museum needs more space for its total, ever-increasing collection.

Cooperation in coal research

John Wilson

Discussions now in progress between the National Coal Board (NCB) and the Office of Coal Research in Washington should lead to closer collaboration between Britain and the United States on research into better ways of using coal. Mr Leslie Grainger, NCB Member for Science, says that an agreement may soon be reached which will allow Britain access to the results of American research.

Naturally, the NCB's position at these discussions would be greatly strengthened if it were undertaking a substantial development programme of its own. The Board needs to set up pilot plants to test processes already studied in the laboratory and if this were done Britain could form a very useful partnership with the United States.

Formal proposals for such development work were to have been made to Lord Carrington, Secretary of State for Energy, but they have been delayed by the general election. The arguments in

favour of setting up the pilot plants are so strong, however, that they will probably be accepted by whichever party is returned to power.

The urgency with which Mr Grainger views these development plans may be judged from the massive increase in the money he thinks they deserve. In November last year he felt that about £20 million would be sufficient for the next five years. Now, barely three months later, he believes that perhaps twice that amount should be spent. That is the sort of money, he says, which must be made available—and quickly—if British industry is to compete in this new technology by the mid 1980s; the Office of Coal Research has already received \$400 million.

The processes which the board wants to see developed to the pilot plant stage include the gasification, liquefaction and fluidised bed combustion of the coal. It has already carried out much of the preliminary research on these projects and American interests have used its facilities. The Environmental Protection Agency, for example, has successfully burnt American coals in a fluidised bed combustion system at the NCB's Stoke Orchard Laboratories, Cheltenham, in some cases paying entirely for specific tests.

The Office of Coal Research, too, has been studying fluidised bed combustion systems at Leatherhead, Surrey, in conjunction with British Petroleum. It hopes to use the knowledge gained here to build a pilot plant at some 30 MW in the United States. The board would like to match this effort with a similar scheme of their own, possibly at Grime-thorpe, Yorkshire, where suitable back-up facilities are already available. This would cost approximately £2 million. Another alternative would be for the board to persuade the Americans to build their larger plant in Britain.

But in any case, Mr Grainger believes that much of the future development of fluidised bed combustion will take place in the United States because American electricity production, unlike British, is expanding. Fluidised bed combustion would thrive on an expansion of generating capacity based on a low grade fuel, and it can also greatly reduce the emission of both sulphur and nitrogen oxides in the flue gases. The keener edge to the environmentalist lobby in the United States and vast reserves of low grade coal combine to make fluidised bed combustion a more attractive proposition there.

The NCB hopes that any technical information gained by the United States in its development of fluidised bed combustion, or any of the other processes, will be re-exported back to Britain. Clearly, this sort of cooperation is the

only sensible way for industrial societies to solve their energy problems.

Family planning: a safer pill

Sally Owen

A new 'pill', containing an ultra-low dose of hormones, is expected to set a new standard of safety for oral contraceptives. Ovranette, which has been medically approved by the Family Planning Association, contains only one-third the amount of hormones in existing low-dose pills.

John Wyeth and Brother Ltd, manufacturers of the new pill, consider the lower hormone content to be an important step forward in oral contraceptive formulation, in line with the recommendation of the Committee on Safety of Drugs in 1969 that oral contraceptives should not contain more than 50 µg of oestrogen.

For some years now a small increase in the risk of blood clotting has been associated with the oestrogen in oral contraceptives. It is felt probable that doses lower than 50 µg a day make this increase even more minute. Following the committee's recommendation several low-dose contraceptives were brought to the market, most of them containing 50 µg of oestrogen.

Ovranette contains 30 µg oestrogen and 150 µg progestogen, which means that a monthly intake of about 11 mg oestrogen is reduced to 3.78 mg. This new pill should reduce the hazards of blood clotting not only because of its low oestrogen content but also because of the low dose and special nature of the progestogen involved.

Ovranette contains a progestogen called norgestrel which is not converted to oestrogen in the body. In fact it is actively anti-oestrogenic, reversing some of the changes brought about by oestrogen. It is thought therefore that the increase in blood clotting will be reduced even further.

One of the problems posed by low-dose oral contraceptives has been the unacceptable levels of breakthrough bleeding or spotting between normal menstrual periods. In clinical trials Ovranette compared well with other combined oral contraceptives, in that the incidence of intermenstrual bleeding was low. The cycle length among trial patients was invariably 28 days, with an almost unchanged menstrual flow. Other side effects, such as feelings of nausea, breast tenderness and depression, were less evident in trial patients who had been taking a pill containing larger amounts of hormones.

news and views

Even small meteoroids are fluffy

EVER since the enormous advances made in radar during the Second World War, scientists have been using radar techniques to study meteors. Most of the pioneer work was carried out at Jodrell Bank, but nowadays the world is dotted with radar research stations busily analysing the echoes returned from the columns of ionisation left behind by ablating meteoroids and relating these echoes to the physical parameters of the particles.

In an important article in a recent edition of the *Journal of Geophysical Research* (78, 8429; 1973), Verniani of the Istituto di Fisica dell'Atmosfera del Consiglio Nazionale delle Ricerche, Bologna, Italy analyses the physical parameters of 5,759 faint radio meteors recorded at Havana, Illinois in 1962 under the Harvard Radio Meteor Project. A system of six radar stations has been operated at Long Branch, Havana at a frequency of 40.92 MHz with a peak transmitted power of around 2 MW. Trains of pulses transmitted from the double trough antenna at the main site were reflected from the electron columns produced by meteoroids in the upper atmosphere (at heights of around 90 km) and observed at a series of five remote sites. These remote sites were connected to the main site by microwave links, the echoes being displayed on oscilloscopes and recorded on film.

The Fresnel pattern produced as the incident meteoroid moves through the antenna beam is used to determine the meteoroid velocity, and the measurement of the echo amplitude yields the electron line density (the number of electrons per unit length along the train). Each of the Fresnel patterns received at the different stations furnishes a value of velocity and line density at a particular time. Velocity can be measured to $\pm 5 \text{ km s}^{-1}$. Deceleration can be obtained from the variation of velocity with time and, by fitting a parabola to the values of line density, the maximum line density and the total number of electrons in the train can be calculated. Measurements of the zenith angle of the apparent radiant and the altitude of the echo points can be used to find the height of the reflection point from the echo range. The deduced ionisation curve gives the beginning and end height of the meteor train. The use of a few assumptions allows the computation of the original mass of the meteoroid when it was outside the Earth's atmosphere, the radio magnitude, the meteoroid density and the ablation coefficient (this coefficient relates the rate of mass loss to the deceleration; it is a function of the heat of fusion of the meteoroid material and the degree of fragmentation. Measurement of the coefficient gives a vital clue to the chemical composition and the physical structure of the meteoroid). It is this multiplicity of data about individual meteors that makes the observations taken at Havana and this work of Verniani's so important in extending knowledge and understanding of these meteoroid particles.

Verniani finds that the 5,759 radio meteors analysed (mean mass of $1.6 \times 10^{-4} \text{ g}$, and radio magnitude $+8.2$) have a mean geocentric velocity of 36 km s^{-1} and decelerate at the rate of 13 km s^{-2} on hitting the atmosphere. These meteoroids produce electron trains of length 11 km containing around 2×10^{16} electrons, the electron line density maximising at a value of $3 \times 10^{10} \text{ cm}^{-1}$. This maximum

ionisation occurs at a height of 92 km, the beginning height being 95 km and the end height of the train 88 km.

How do these observations tie in with other work on meteors? First, the mean density of the meteoroids is found to be 0.8 g cm^{-3} , and even more important this density is independent of mass in the range 10^{-6} to 10^{-3} g , suggesting that meteoroids have a porous, loosely conglomerate structure similar to that found for photographic and visual meteoroids having masses 10^6 times greater (see Verniani, *Space Sci. Rev.*, 10, 230; 1969). This favours a cometary origin for these particles as opposed to an origin as asteroidal collision debris, a process which would produce particles of much higher density. Verniani also finds that sporadic and shower meteors are very similar, indicating that the cometary origin of most meteoroids extends right down to 10^{-6} g .

Does the low value for the meteoroid density provide an indication of the structure of comets? The 'gravel bank' model of Lyttleton (*Comets and their Origin*; Cambridge University Press, 1953) has comets consisting of a cloud of widely spaced, small, rocky particles whereas Whipple (*Astrophys. J.*, 111, 375; 1950) postulates that comets have nuclei, these being a conglomerate of dust particles bound together by solid ices. Solar heating leads to the formation of a porous dust crust surrounding a central dirty iceball; this crust then breaks up irregularly to produce meteoroids, the low densities of the meteoroid being caused by their spongy texture, the holes in the sponge having been filled with ice when they were in the cometary nucleus. So the low density can be easily accounted for by Whipple's model; with the 'gravel bank' one would have to postulate that the gravel was itself in a low density form and this would have repercussions on the ease with which comet tails are formed. These particles, however, could be easily produced if comets are accreted from interstellar clouds by gravitational focusing. The constancy of density with mass indicates that micrometeoroids, particles of masses less than 10^{-6} g which by radiating away their heat energy float to ground and do not ablate, could be 'fluffy' and of low density whereas previously these particles were thought to have densities around 3.5 g cm^{-3} , the larger radio and visual meteoroids simply being made up of collections of these micrometeoroids.

The low density value for radio meteors is further supported by the results obtained by Verniani for the train length. The mean train length of 11 km is much shorter than was theoretically expected when meteoroids were thought to be solid, compact, stone or iron particles which simply dissipated the energy obtained from the impacts of individual air molecules by surface vapourisation of the meteoric material. The short train values lead Verniani to the conclusion that these small radio meteors often fragment, this being the same conclusion that Jacchia drew from his observations of faint photographic meteors and that Jones and Hawkes (*Nature*, in the press) draw from their work on very faint (8th magnitude) "image intensifier plus television" meteors observed at the University of Western Ontario. It seems that all meteoroids between the mass limits of 100g and 10^{-6} g are porous, fragile, crumbly objects made up of loosely conglomerate spongelike material; they all originate in comets and it is to be expected that the mean velocity of the meteoroids is independent of mass, a fact which unfortunately cannot be checked with data from radio meteors because of the height-ceiling selection effect inherent in the technique.

D. W. H.

Synthesis of ideas on early man

from a Correspondent

IN New York recently an attempt was made to bridge the gap between the palaeontologists who find and describe hominid fossils and those who seek to interpret them. A week-long conference (January 26 to February 2) was sponsored by the National Science Foundation to deal specifically with African Plio-Pleistocene hominids, the aim being to consider the fossil evidence, identify the outstanding problems and to formulate research strategies.

The first part consisted of public presentations of the evidence by the discoverers of the fossils. W. W. Bishop (Bedford College, London) began by presenting the chronological and faunal framework for the East African sites. The physical data on chronology, both isotopic and palaeomagnetic, combine with faunal correlations to provide a formidable backcloth for the hominid remains. He emphasised the primary, but not always infallible role of physical dating methods; it was the incompatibility of the fauna with the early dates from Kanapoi in Kenya that led to an eventual revision of the isotopic dating evidence. The situation in East Africa is in sharp contrast to that in South Africa where, although faunal correlation data are being accumulated,

there is a total lack of physical data on chronology.

There was unanimous scepticism about the use of geomorphological evidence to date the South African cave sites. It was felt that uncertainties about the dates of inception of planation cycles, the conflicting evidence from deep-sea cores, documentation of highly variable erosion rates and the influence of various lithologies on nick-point progression make such estimates, based on geomorphology, unacceptable as primary evidence. Hopes for adequate dating of these sites currently rest on fission track and palaeomagnetic studies. Bishop also presented the hominid remains from the Baringo basin (Kenya) and B. Patterson (Harvard University) wittily explained the context of the Kanapoi and Lothagam sites, and in doing so showed a healthy reluctance to elevate his hominid fossils to a position above that of the other fauna. He told of how the Kanapoi site was found as the result of a 'Sunday excursion' for which his field expeditions are apparently notorious.

M. D. Leakey (Olduvai Gorge) showed, albeit unintentionally, what a profound influence the painstaking archaeological, palaeontological and geological studies at Olduvai have had on the standard of field and laboratory work at other sites. F. C. Howell (University of California, Berkeley) and R. E. F. Leakey (National Museums

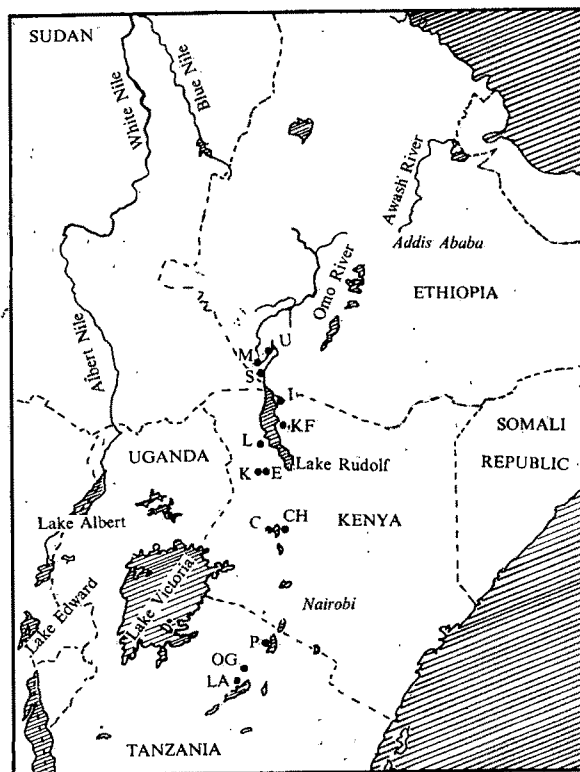
of Kenya) showed how successful this sound methodology can be when applied to the extensive fossiliferous exposures in the lower Omo Valley and in the area east of Lake Rudolf. There is apparently at Omo, as at Olduvai, evidence for an environmental change about midway in the sequence and this may be an explanation for some of the inconsistencies that exist in the isotope dates and the faunal evidence between Omo and East Rudolf.

The evidence from the South African cave sites was reviewed by C. K. Brain (Transvaal Museum) in a presentation that was perhaps the highlight of the meeting. Recent excavations at Swartkrans have revealed a new stratum, the orange breccia, and evidence of solution channels in the outer cave breccia. It is now known that SK15, one of the 'Telanthropus' mandibles comes from this channel fill and is probably not contemporaneous with the other hominid fossils. The possibility that other hominids were associated with similar channels may vitiate the importance of the South African hominids as being a sample from a limited time range and thus being better indicators of early hominid variability than the more temporally separated East African sample. Brain's elegant, and beautifully illustrated experiments using captive cheetahs and the companion investigations of leopard lairs may explain the curious differences in skeletal preservation between bovids and primates at the cave sites.

The recently prospected fossiliferous exposures in the Awash Valley in north-east Ethiopia were described by D. Johanson (Case Western Reserve University). Faunal correlations indicate an age of about 3 million years for the exposures at Hadar which have yielded hominid leg bones and a cranial fragment. Fragmentary though some of these specimens are, the excellent preservation of other vertebrate fauna suggests that these will not be the last hominids found at Hadar.

The remaining four days were held in the comfort of the Wenner-Gren Foundation. Sessions were devoted to morphology, demographic aspects, cultural evidence, patterns of diversity and implications of taphonomic evidence for behaviour patterns. Of the current research problems differences in methodology were the most evident. Views polarised into those who considered that it was necessary to establish patterns of morphological variability before phylogenetic and functional interpretations of the hominids could proceed. Others considered that examination of the material should be preceded by hypothesis formation about broader patterns of hominid behaviour and variability: suffice it to say that this

Map of some of the Plio-Pleistocene hominid localities in eastern Africa. M (Mursi), U (Usno), S (Shungura) Formations, lower Omo basin; I, Ileret sector; KF, Koobi Fora sector of East Rudolf area; L, Lothagam; K/E, Kanapoi and Ekora of lower Kerio drainage; CH, Chesowanja, Baringo basin; K, Kanam; P, Peninj, Natron basin; OG, Olduvai Gorge.



matter was not resolved! Opinions differed about the role of multivariate statistics, the criteria for establishing population diversity, the association of culture with any particular hominid group, the interpretation of bone 'tools' and the significance of differences in endocast morphology; in fact few 'stones' were left unturned.

Despite differences in approach, perhaps an uncomfortable level of broad agreement was reached about the existence of cranial, dental and postcranial morphotypes (though one suspects that for some participants there were too many cranial morphotypes to fit the teeth or postcranial material!). Future research problems that were identified include more comparative work on endocranial morphology, dental wear patterns and postcranial variability and further research into the implications of dental eruption patterns of hominines and current taphonomic studies for hominid behaviour.

This conference successfully brought together workers whose research approaches are different, but whose common aims are to discover more about the structure and behaviour of early man. This meeting complemented the workshop conference held in Nairobi in September 1973, which attempted to synthesise the results of work at Omo and East Rudolf. The publications resulting from these two meetings will attest to the growing scientific maturity of hominid studies and show the success of the international and multidisciplinary approach to hominid palaeontology which has been a feature of the research both at Omo and at East Rudolf.

Are accretion disks stable?

from a Correspondent

THE stability of accretion disks in binary X-ray sources comes under critical review in an article by Lightman and Eardley in a recent issue of *Astrophysical Journal* (187, L1; 1974). The accretion of material by a compact object (a neutron star or a black hole) from its companion in a binary star system is thought to be the main source of power for the luminous X-ray sources that have been discovered within our galaxy during the past few years. Because of the rotation of the binary system, transferred material is forced by the conservation of angular momentum initially to form a disk around the compact object. Differential rotation and viscosity, in the disk, transfer angular momentum outwards, allowing the matter to spiral inwards, liberating its gravitational potential energy as it does so. If the compact object is a neutron

star, most of the energy is liberated at the surface of the star itself, where the accreting material crashes into it. But, if the compact object is a black hole, then all the luminosity comes from the disk, and the matter arriving at the inner edge of the disk is swallowed quietly by the black hole. Thus for accretion by a black hole, widely speculated to be the case for the X-ray source Cygnus X-1, the stability of the accretion disk is an all important matter.

The most uncertain element in all models of accretion disks is the viscosity—ordinary molecular viscosity is far too small, and authors usually invoke turbulence or magnetic fields. A common (*ad hoc*) assumption is that the effective kinematic viscosity (with dimensions of length²/time) is roughly equal to the vertical thickness of the disk (the supposed size of a typical turbulent eddy) times some constant fraction of the sound speed (since supersonic turbulence would be rapidly dissipated). The difficulty arises in the inner regions of the disk where radiation pressure, rather than ordinary gas pressure, dominates the vertical structure. In this region, it is unclear what the 'sound speed' relevant to turbulent motion should be, and it is usually assumed to be that dictated by the radiation. Lightman and Eardley, however, have demonstrated that, in this part of the disk, this assumption about the viscosity is not consistent with the disk being in a steady configuration. They show, moreover, that if the disk is forced initially to conform to the previously calculated 'steady' configuration, it evolves secularly, breaking up into rings. The reality of these rings is, however, questionable, since their formation depends on being able to start in a supposed 'steady' situation, which has already been shown not to be self-consistent.

The reason for this 'instability' is fairly simple. The assumptions mentioned earlier lead to the conclusion that in this part of the disk, the viscous stress is inversely proportional to the surface density of the disk. Thus, if a small region of the disk has a lower than average surface density, the viscous stress, and hence the radial velocity, there will be higher than average, and so the surface density will decrease still further.

The authors suggest two possible alternatives. First, if one assumes rather than the 'sound speed' relevant to turbulent viscosity is that given by the gas pressure, then the resultant steady accretion is self-consistent. Second, if the assumptions made are roughly correct, then the idea of a steady accretion disk must be abandoned. The observed variability of the source Cygnus X-1 on timescales as short as milliseconds might lead credence to this idea. In any

event this contribution serves as a timely reminder to theoretical astronomers that '*ad hoc*' assumptions which seem to work should not have too great a trust put in them.

Darwin glass related to tektite fall?

from our Geomagnetism Correspondent

THE material that has come to be known as Darwin glass was first recognised and described just 60 years ago by Suess who found large quantities of it along a north-south track some 3 km to the east of Mount Darwin in Tasmania. About 50 years later, geochemical studies and the discovery that the glass contains coesite led to the view that the Darwin glass must have been formed by impact—a conclusion which was strikingly confirmed two years ago when Ford (*Earth Planet. Sci. Lett.*, 16, 228; 1972) discovered Darwin Crater, a circular depression with a diameter of about 1 km lying some 4 km or so to the east of the most southerly of Suess's deposits.

It is now known that there are far more examples of glass in the vicinity of Mount Darwin than Suess had realised; and with the aim of confirming the genetic relationship between the original occurrences and the more recently discovered crater glasses, Gentner *et al.* (*Earth Planet. Sci. Lett.*, 20, 204; 1973) have used both potassium-argon and fission track methods to date glass samples from the interior and rim of the crater and from areas up to 2 km away from the rim. The mean potassium-argon and fission track ages are 0.70 ± 0.08 and 0.74 ± 0.04 million years, respectively, giving a combined age of 0.73 ± 0.04 Myr. This compares with the fission track age of 0.72 ± 0.02 Myr for the longer-known glasses, obtained previously by Gentner *et al.* (*Geochim. cosmochim. Acta*, 33, 1075; 1969).

Gentner and his colleagues thus conclude that all the known Darwin glasses were produced at the time of the impact that formed Darwin Crater 0.73 Myr ago. But there may be more in this than simply a study of the relations between glass deposits. That the Darwin glasses occur geographically close to the Australasian tektites has been obvious for some time; and what Gentner *et al.* have now done is to show that the glasses and the tektites have the same age. This is apparently the first evidence obtained that an impact crater was formed at the time of the Australasian tektites. It seems hardly likely that this is a coincidence; and yet it is unlikely that an impact forming a crater as small as the Darwin Crater could be

responsible for the large Australasian tektite field. One reconciling conclusion is that the meteorite or comet fall (if such there was) leading to the formation of the Australasian tektites produced a number of craters, of which the Darwin Crater is but one. If so, a careful search in the area should reveal them in time.

No role yet for poly (A)

from our
Molecular Genetics Correspondent

ONE of the most intriguing questions in the area of messenger RNA production in eukaryotic cells is the role of the sequence of polyadenylic acid (poly (A)) which almost all the messengers seem to carry at their 3' end. A sequence of about 200 residues of adenylic acid is added, probably one base at a time, to the 3' end of the apparent mRNA precursor, heterogeneous nuclear RNA (Hn RNA), after or during its synthesis from chromatin. After cleavage of a 3' terminal sequence containing the poly (A), transport to the cytoplasm is succeeded by association with ribosomes for translation.

One speculation about the possible role of poly (A) is refuted by the results reported by Bard, Efron, Marcus and Perry in the February issue of *Cell* (1, 103; 1974). The idea that the poly (A) might be necessary solely for nucleocytoplasmic transport has previously been rendered less likely by observations that it is found in tumour viruses whose expression is confined solely to the cytoplasm and also in mitochondrial messengers where no transport is implicated. One inference which might be drawn from these results is that the poly (A) is implicated in some role in translation, perhaps controlling the stability of mRNA or its capacity to act as template for the ribosomes. Although the sequence of poly (A) on a messenger seems to shorten progressively with time passed in the cytoplasm, studies of mRNA turnover showed that this is not correlated with any increase with time in the probability of mRNA degradation.

The possible role of poly (A) in controlling translational capacity has therefore been examined by Bard *et al.*, using two types of experiment: in the first, they examined the abilities of messengers bearing different lengths of poly (A) to be translated within the mouse L cell; and in the second they compared the activities as templates *in vitro* of mRNAs either possessing or stripped of their poly (A) sequences.

Old and new mRNAs can be preferentially labelled in the L cell cytoplasm by labelling with ^{32}P , for 20 h but with ^3H -adenosine for only 1.75 h. When

polysomes are then extracted and analysed, the ^{32}P label identifies older messengers whereas the ^3H label is found in newly synthesised messengers. The new messengers possess a length of poly (A) of about 180 residues, but in the old messengers its length is much reduced and may be as small as 50 bases. Bard *et al.* found that there is no difference in the ratio of new to old mRNA in various polysomes fractions, implying that both classes of mRNA share the same translational capacity.

An alternative possibility, that overall translational efficiencies are different but that the same ratio of initiation to elongation is maintained, was excluded by experiments utilising temperature shock. This treatment disaggregates polysomes by preventing initiation of protein synthesis; ribosomes therefore run off their current messengers to release free messenger ribonucleoprotein. Lowering the temperature allows the polysomes to reform by utilising the released mRNP. After labelling old mRNA by incubating cells for 2 h with ^3H -uridine followed by a 21 h chase, and labelling new mRNA by incubation for 1 h with ^{14}C -uridine, Bard *et al.* found that reassembled polysomes display a ratio of new:old mRNA very close to that of the control. The capacity to initiate protein synthesis of both classes of messenger must therefore be virtually identical.

Because these experiments compare messengers possessing different lengths of poly (A), Bard *et al.* then turned to *in vitro* experiments to compare messengers with or completely without poly (A). By using a 3'-OH exoribonuclease to degrade the poly (A) sequence, and by then utilising only messengers which had retained their original size (to exclude those suffering other breaks), they isolated a messenger fraction identical to that usually extracted from L cells, but lacking poly (A). No difference could be found in the translational capacity of the deadenylated mRNA compared with the messengers possessing poly (A), using an *in vitro* system for synthesising proteins derived from wheat embryo.

Poly (A) therefore seems to lack any role in controlling the activity of messenger RNA in protein synthesis either *in vitro* or *in vivo*. The ubiquity of poly (A) in eukaryotic messengers (the only well established fraction lacking it is that coding for the histones) implies that it must have some important function. Previous experiments have excluded control of messenger lifetimes as its function, leaving it with no role apparent at the level of translation. Although preventing the addition of poly (A) halts the production of mRNA from HnRNA, the presence of poly (A) in messengers not derived from the nucleus suggests

that it must have some cytoplasmic function. No reconciliation of this paradox is evident at present: poly (A) remains a sequence in search of a function.

Inside the sickled red cell

from a Correspondent

CURRENT interest in sickle cell anaemia itself and in the behaviour of deoxy sickle cell haemoglobin was the subject of recent comment in these pages (see *Nature new Biol.*, 242, 33; 1973). This interest has been stimulated, partly by increased support in the United States for work on the clinical management of patients with sickle cell anaemia and tests of possible therapy, and partly by new insights into the aggregation of deoxy Hb-S from a variety of techniques, in particular electron microscopy and fibre type X-ray diffraction studies. There is now a considerable literature on the aggregation of deoxy Hb-S into an ordered phase, starting with the birefringence studies of Sherman in 1940, even before the recognition of Hb-S as a haemoglobin variant.

One of the latest additions to this literature is an optical study by Hofrichter, Hendrickson and Eaton (*Proc. natn. Acad. Sci. U.S.A.*, 70, 3604; 1973) on the orientation of deoxy Hb-S molecules within the long straight fibres which have been shown by electron microscopy to make up the ordered phase. The fibre model proposed by Finch and colleagues (*ibid.*, 70, 718; 1973) is a microtubule composed of stacked rings of six Hb-S molecules. Since each ring is rotated slightly, relative to the preceding ring, the structure can alternatively be envisaged as six intertwined helical filaments, each having about 48 Hb-S molecules per turn. Neither electron microscopy nor X-ray diffraction has as yet provided definite information on the orientation of the molecules within the fibre, or the role of the mutation sites (Glu A3(6) β \rightarrow Val) in the intermolecular contacts.

Eaton and his colleagues have made polarised absorption measurements on single sickled red cells and single crystals of deoxy Hb-S. The polarisation ratio of sickled cells gives a lower limit for that of an individual fibre, and taken in conjunction with the absorption properties of the haemoglobin molecule restricts the direction of the long molecular axis (z) to within 22° of the fibre axis. The highly anisotropic absorption ellipsoid of the haemoglobin molecule required for this work was calculated for the Soret band, since optical studies on haem protein single crystals by Eaton and colleagues had shown this haem chromophore transition to be nearly perfectly in-plane. The haemoglobin ellipsoid was

obtained by summing the squared projections of all the haem planes on to the form ellipsoid of the molecule, and this was then transformed into the crystallographic system.

After defining the limits of the orientation of the x axis, and using the Finch stacked-ring model for the molecular positions, with the requirement that at least one mutation ($\beta 6$) site is part of an intermolecular contact, the measured polarisation ratio for the Soret band requires that the true molecular dyad (y)

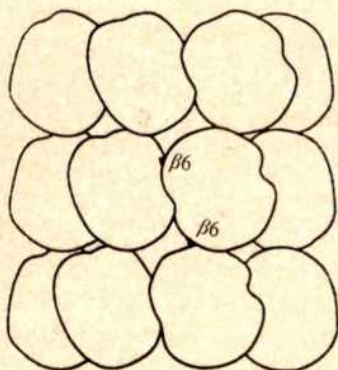


Diagram of one of the three classes of model for the orientation of molecules in the sickle cell fibre allowed by the optical results of Hofrichter *et al.* and by the additional constraint that at least one $\beta 6$ site is part of an intermolecular contact. If the maximum possible tilt of the x axis is used, the other $\beta 6$ residue of a molecule is in a position to make an interring contact.

axis passes through some part of an adjacent molecule in the same ring of six molecules. This range of orientation for the y axis turns out to be approximately perpendicular to those proposed in previous models.

The new information obtained from the optical studies allows three classes of model to be considered, which differ in respect of the possible dispositions of the $\beta 6$ sites. In all three models a $\beta 6$ residue could assist in stabilising intraring contacts. If the maximum possible tilt of the x axis is used, the other $\beta 6$ residue of a molecule is in a position to make an interring contact, though more extensive polarised absorption measurements will be required to establish this interesting possibility. In any case the absorption polarisation work, which is technically very impressive, has already provided new insights into the detailed structure of the deoxy Hb-S fibre and may suggest new approaches to the practical problem of inhibiting the aggregation process.

Not all at sea on the Channel

ON Thursday, January 31, a large contingent of French scientists converged

upon London to meet their English counterparts for two days of discussion on the geology of the English Channel. Although physical constraints required that the scientists meet in either London or Paris, metaphorically they met half way—a great deal of academic *entente* was evident throughout the proceedings. The meeting, jointly organised by the Royal Society and the Marine Studies Group of the Geological Society, was a great success. And it was not without its surprises: "I began", said one of the organisers, "by asking for volcanoes, and in the end I was given ice".

But despite the more surprising aspects, the meeting showed that a new understanding of Channel geology has emerged which will not only have important implications in future studies of the geology of north-western Europe, but which may well result in a considerable reappraisal of established theories concerning that area. This is largely due to the amount of detail about the basement geology which has been collected by the French scientists.

There is evidence to indicate that the region has had an important tectonic history, the effects of which have not been fully appreciated in the past. There have been two major phases in the history of the Channel, probably interspersed with many minor tectonic events. A. J. Smith (University College, London) believes that both phases are intimately associated with events in the history of the spreading Atlantic Ocean. Soon after the end of the Hercynian orogeny about 280 million years ago, the Atlantic began to open along a trend aligned with that of the Channel, and many of the primary structures of the Channel originated at this time. During the Early Cretaceous, probably about 130 m.y. ago, the second phase developed as the Bay of Biscay opened and the axial trend of the Atlantic changed to its present orientation.

Consideration of the present structure and geology of the Channel shows that its tectonic history must have had some influence on the geological evolution of northern France and southern Britain. The Channel can be divided into three areas on a structural basis: an area to the west of the Anglo-Norman Ridge; a central area between this ridge and the Bembridge-St Valery Line; and a third area to the east. It is as a result of studies of the central area that geologists have come to appreciate the importance of the Channel's tectonic history. Within this central region two east-west structures downturn beds to the south. Geophysical data indicate that the tectonic activity in the Channel has been largely restricted to vertical block movements of the basement (which to some extent are still continu-

ing), and the surface structures of the central area are probably reflections of the basement structure. But, the important fact is that the southernmost of these two structures joins the Bembridge-St Valery Line and becomes deflected onto the French coast. The deflected structure has become a very minor feature by the time it reaches the coast, but, significantly, it is in perfect alignment with major structural features which pass through northern France, and into the Massif Central. Furthermore, the northern extension of the Bembridge-St Valery Line passes into important structures in south Dorset.

The implication is that major structural features which have previously been interpreted without any consideration of the role of the events which are manifest in the Channel, will now have to be examined more closely. Are the Mendips really just part of the Hercynian Front, or do they in fact reflect the vertical movements of the basement rocks of this area? And was the Weald really folded into its present structure by epeirogenic movements during the Alpine orogeny; or is D. Curry (University College, London) correct in the reiteration of his old idea that the Hampshire, London and Somme basins did not form separately?

But despite the implications raised by these issues it was largely the well-received theory of G. A. Kellaway (Institute of Geological Sciences, London) and his colleagues which dominated the discussions. Early on in the meeting Smith had suggested that there may have been considerable volcanicity accompanying the first major tectonic phase, and that the lavas which accumulated may be responsible for the present isostatic balance which leaves the western area of the Channel as a basinal depression. Unfortunately none of the other speakers could provide proof for this attractive hypothesis, and so Kellaway, unable to provide a volcano, provided a glacier instead. He has managed to find an impressive amount of evidence, including boulder clay from the Slinden Pipes, to show that during the Anglian glacial advance the Channel was filled with glacial ice which moved eastwards from the edge of the continental shelf, and deposited the abundant erratics which today litter the coastline of southern England.

Kellaway also believes that the 'raised beaches' around the Sussex coastline are not raised beaches at all, but are marginal outwashed deposits. His theory could certainly provide an acceptable explanation for the previously mysterious buried channels and valleys and for the Hurd Deep, all of which may be the result of subglacial erosion.

Accuracy of tree-ring dating

from our Archaeology Correspondent

THE correction of the radiocarbon time scale by means of tree-ring dating has profoundly affected archaeological chronologies, and has important geophysical implications. Hitherto, the calibration has been based upon a single 7,400-year tree-ring sequence, established for the bristlecone pine by Ferguson. The production of an independent tree-ring sequence for the same species, extending back to 3535 bc (LaMarche and Harlan, *J. geophys. Res.*, 78, 8849; 1973) is thus of considerable importance. That it should differ by only two years from the original Ferguson sequence offers strong corroboration to both.

In both cases the trees examined are in the southern White Mountains of California. In the new study wood from a limited area on Campito Mountain, at elevations over 3380 m, was used; Ferguson's earlier study (page 237 in *Radiocarbon Variations and Absolute Chronology*, edit. by Olsson; Almquist and Wiksell, Stockholm, 1970) was based on wood from a lower elevation at Methuselah Walk, about 10 miles south of Campito Mountain. The Campito sequence is based on samples from 37 living trees, which take it back to ad 600, and woods from 70 dead trees were utilised to carry the sequence back to 3535 bc. There are three potential error sources in such a count. To the risk of extra intrannual rings is added that of locally absent rings. And, third,

there is the possibility of the mismatching of rings from one specimen to the next as the record is built up. LaMarche and Harlan conclude that false latewood and 'earlywood' bands can be recognised by their cell structure, so that the first problem is not difficult. They offer arguments to show that when there is a possibility of a few annual rings being uncounted in the chronology (that is of missing rings), "the error in the chronology from this source should only be of the order of one year". Cross-dating errors can be controlled by the recognition of characteristic frost damage rings, often shared by the different trees in a specific area in very cold years.

The importance of the work is that these considerations were put to the test by a detailed comparison of the Campito Mountain chronology with the Methuselah chronology. The cross dating is demonstrated objectively by means of cross-correlation analysis. This was first carried out using the dates originally assigned to the Campito chronology, and the coefficient obtained had negative or low values for part of the period. When the Campito dates were adjusted by insertion of two missing rings, the cross-correlation coefficients for the earlier period were positive and highly significant. The authors conclude: "The incidence of false rings that might erroneously increase the age is so small as to preclude explicit evaluation. The only important potential source of error seems to be locally absent rings. On the basis of the close agreement of the estimated number of rings absent from

the Campito chronology with the observed discrepancy between the Campito and Methuselah chronologies, it is highly probable that the absolute error of the Methuselah chronology at 3435 bc is, in fact, zero".

This conclusion is of fundamental interest to archaeologists and others who seek to use the dendrochronological calibration of radiocarbon, because hitherto this has been based principally on the Methuselah sequence. Although the accuracy of this sequence has not seriously been questioned, independent corroboration was desirable. Sceptics could yet argue that sequences obtained from trees of the same species, from locations only 10 miles apart, are not necessarily independent, but the results must strengthen the bristlecone pine sequence.

They cannot help, however, with two other problems of the calibration. In the first place there is the possibility that as a result of the diffusion of recent tree sap across older tree rings, the radiocarbon determination obtained from a given ring may yield a date substantially later than that of the ring's formation (Berger, *Phil. Trans. R. Soc. A*, 269, 33; 1970). And, second, the atmospheric concentration of radiocarbon in southern California may not always have been the same as, for instance, in Europe. These two problems can only be resolved by tests outside California. The long tree-ring sequence (mainly of oak) at present being established in Belfast may ultimately answer both questions. Meanwhile, comparison of radiocarbon dates obtained from dendrochronologically-dated bristlecone pine samples with radiocarbon dates obtained from archaeologically and historically dated samples from ancient Egypt at least produces no conflict. But the errors associated both with the radiocarbon determinations and with the Egyptian archaeological contexts are so large that this comparison is far from conclusive.

A problem related to these is that of the shape of the curve which relates calendar and radiocarbon dates. The version originally prepared by Suess had many kinks (page 303 in *Radiocarbon Variations and Absolute Chronology*). Other laboratories, notably at the University Museum in Philadelphia and the University of Arizona, have used smoothing procedures to give a more convenient curve, although as Burleigh has pointed out (*Antiquity*, 47, 309; 1973) there is at present no sound reason for eliminating the kinks.

This question of the precise shape of the curve is, however, secondary: the primary problem is the general and worldwide validity of the data upon which it is based. The new Campito sequence serves to confirm one of its



Standing snags of bristlecone pine on Sheep Mountain in the Campito Mountains of California. These trees have been

dead for more than 300 years. (Photo: Laboratory of Tree-ring Research, University of Arizona.)

sustaining arguments, and is thus a first step towards the validation of the calibration as a whole.

Pion capture reveals alpha clusters

from our Nuclear Theory Correspondent

WHEN a negative pion comes to rest it is captured by a nucleus and gives it all its rest energy. Since energy and momentum must be conserved in the capture process the pion cannot be captured by a single nucleon but must interact with several nucleons at once, so a detailed study of the particles emitted provides information on nucleon correlations in the nucleus.

One of the most likely forms of nucleon correlation is their tendency, especially in light nuclei, to form α clusters, and a recent experiment by Lewis, Ullrich, Engelhardt and Boschitz (*Phys. Lett.*, **47B**, 339; 1973) on the capture of negative pions by ^{16}O provides new evidence of the presence of these clusters in nuclei.

They looked at the γ -ray spectrum emitted by a ^{16}O target irradiated with slow pions and found a strong peak due to the 4.44 MeV γ -ray from the decay of the first 2^+ excited state of ^{12}C . It is thus natural to suppose that the pion capture has broken the ^{16}O into an α particle and an excited ^{12}C nucleus, and indeed it is known that this mode of breaking up is about six times more likely than the corresponding process leaving ^{12}C in its ground state.

Since the α particle has a momentum distribution relative to the ^{12}C in the original oxygen nucleus, the recoiling carbon nuclei have the same distribution, superposed on the recoil momentum determined by the rest mass of the pion. This momentum distribution produces a Doppler broadening of the shape of the γ -ray line, and measurement of this broadening gives the momentum distribution of the α particles.

This momentum distribution can also be calculated from a simple harmonic oscillator model, thus providing a check on the interpretation of the data. In such a model, the momentum distribution of a particle moving with angular momentum L with respect to the centre-of-mass of the harmonic oscillator potential has the form $P(K) \propto K^{2L} \exp(-K^2/2Q_0^2)$, and fits to the data given $Q_0 = 75 \pm 5 \text{ MeV/c}$. This may be compared with analyses of the $^{16}\text{O}(p, \alpha)$ knockout reaction that give $Q_0 \approx 75 \text{ MeV/c}$. The values of Q_0 and Q_2 are expected to be similar, so this is an indication of the consistency of the two analyses.

This experiment essentially gives the distribution of the sum of the momenta

of the four nucleons emitted when pions are captured by ^{16}O . These four nucleons are most probably combined to form an α particle because the alternative interpretation of absorption on a deuteron followed by evaporation of two nucleons would give the characteristic γ -rays from the decay of ^{12}B ; and these are not observed. Other possible origins of the observed γ -rays were excluded by repeating the experiment with different targets.

It is thus very reasonable to interpret these observations by the α particle absorption model and this gives further support to the idea that α clusters are present in nuclei. Similar results have been obtained for other nuclei besides oxygen.

Population energetics of kangaroo rats

from our Animal Ecology Correspondent

It has been suggested that because terrestrial herbivores have low efficiencies of consumption of net primary production (NPP), their populations generally are not limited by food (Slobodkin *et al.*, *Am. Nat.*, **101**, 109; 1966; Wiegert and Owen, *J. theor. Biol.*, **30**, 69; 1971). Most species of small mammals from the temperate biomes consume between 0.1% and 1.0% of the NPP annually. When this amount is expressed in terms of available productivity (AP)—that is, ignoring inedible parts of the plant—the values range from 0.1% to 4.0%, with a few exceptions (Chew and Chew, *Ecol. Monogr.*, **40**, 1; 1970; Wiegert and Evans, in *Secondary Productivity in Terrestrial Ecosystems* (edit. by K. Petrusewicz) 499, *Polish Academy of Science*, Warsaw, 1967; Grodzinski *et al.*, *Acta Theriol.*, **11**, 419; 1966). Sohlt has now published some interesting data on the impact of a population of the kangaroo rat (*Dipodomys merriami*) on the Mojave desert and shows that the rats take 6.9% of the NPP and 10.7% of the AP annually (*Ecol. Monogr.*, **43**, 357; 1973). The study offers some thoughts on why population densities of desert rodents are as high as they are.

Sohlt estimated population density by recapture trapping and the Lincoln Index. During the year of study the density averaged 16.2 rats ha^{-1} , a high figure for a species living in conditions where the NPP is just 1,400 megacal $\text{ha}^{-1} \text{yr}^{-1}$. (In temperate zones the NPP is between 20,000 and 30,000 megacal $\text{ha}^{-1} \text{yr}^{-1}$.) Sohlt measured resting energy expenditure, the energy cost of activity, the energy expense of growth, assimilation efficiency and calculated the total energy flow (E) through the population to be 85.5 megacal $\text{ha}^{-1} \text{yr}^{-1}$.

From his studies on growth and reproduction he deduced that the secondary productivity (P) of the population was 0.7 megacal $\text{ha}^{-1} \text{yr}^{-1}$, or 0.8% of the total energy flow. This is somewhat lower than the P/E ratio for other rodents which fluctuates between 1.2 and 3.0%. This suggests that kangaroo rats are not adapted for utilisation of as high a proportion of their total energy flow for secondary productivity as are non-desert species. Perhaps this adaptation is prudential in an environment where the amount of food production is unpredictable.

Although several species of plants were available to the rodents, they showed a strong preference for the annual storksbill *Erodium cicutarium*. Seeds formed the major item in the diet, but leaves were consumed during the winter. Laboratory studies showed the assimilation efficiency (AE) to be 0.87. Assuming this, the amount of food that had to be consumed such that E/AE equaled 85.5 megacal $\text{ha}^{-1} \text{yr}^{-1}$ was calculated to be 97.8 megacal $\text{ha}^{-1} \text{yr}^{-1}$. A little more than 1.0 megacal was supplied by arthropods; most of the rest by *Erodium*.

Further field studies revealed that, in order to obtain this calorific input chiefly from one species of plant, the rats consumed, incredibly, more than 95% of the *Erodium* seed production and more than 90% of the total *Erodium* production. The population thus exerted a massive effect on the energy turnover of this one species, although at 6.9% of the total NPP it had little effect on the vegetation as a whole. Its impact on the survival chances of this species population would be immense if the remaining 5% of the seeds are insufficient to support as great a plant population in the succeeding year. It is a pity that the study was not continued to ascertain what were the population levels of rat and storksbill during the following year. Populations of desert annuals are limited by rainfall so that desert rodents are unlikely to exert an important controlling effect. If rainfall was not limiting, the rodents would likely consume *Erodium* until it became scarce to find and in that way actually limit its population.

Sohlt's study shows that this population of *Dipodomys* lived dangerously on the brink of overexploiting its food resources. Wiegert and Owen suggested that low consumption of plants avoided long term oscillations of the plant populations. In deserts, where extremes of environmental factors make oscillations unavoidable, the evolution of such a damper would prove ineffective. Thus there is no disadvantage to a population of rodents consuming more than 95% of its major food source.

Glassy state good for lasers?

from our Solid State Physics Correspondent

LASER beams are often of such high intensity that the electric component of the electromagnetic wave reaches the level of the breakdown field for the medium through which it is travelling. In solids, local fractures and other damage are seen at a level of about 10^8 V m⁻¹ and in gases, plasma discharges are formed. The instantaneous field at which this takes place in solids is called the intrinsic optical damage field (the word 'intrinsic' is necessary because in solids there are other damage phenomena which are not intrinsic to the material and which are apparently caused by increased energy deposition in small imperfections in the transparent materials; these effects may occur at much lower light intensities than the electrical breakdown).

The intrinsic breakdown is interesting both from the fundamental and from the practical point of view. Electron avalanching is almost certainly the mechanism by which damage occurs and the processes by which hot electrons impart energy to a solid lattice is of great interest at present. Also, in the bid to make more and more powerful lasers, materials with the highest possible tolerance to the extremely high frequency electric fields present in the light pulse have to be found or fashioned; of special interest, of course, are the optically transparent glasses, mainly amorphous compounds with a wide band gap such as that possessed by many oxides. Here, the question of electron transport in a disordered lattice presents a double problem; first, because the theory of high field transport in any material with a wide band gap is not yet well developed and, second, because the theory of carrier scattering in disordered systems is more complex than that in crystalline lattices and is also in an embryonic state; some courageous attempts at predicting breakdown from data on lattice vibrational modes in an amorphous dielectric have, however, been made, with fair success (Lynch, *J. appl. Phys.*, **43**, 3274; 1972).

Thus the need for utilitarian data on the behaviour of glass optical elements in laser systems leads into a distinctly challenging area of research and leaves one to sink or swim, and perhaps to grasp at straws of data as they come past. Such a straw, perhaps, is a first report of a comparison of intrinsic damage fields in some glasses, some polycrystalline solids and some comparable single-crystal materials by Fradin and Bass, of Raytheon Research and the University of Southern California, re-

spectively (*Appl. Phys. Lett.*, **23**, 604; 1973).

The comparisons are between quartz and fused silica, polycrystalline and single-crystal potassium chloride and a germanate glass and its crystalline form. First, the fused silica did not show breakdown until a light intensity of 108 GW cm⁻² was reached, five times that at which crystalline quartz broke down. This factor of five in intensity corresponds to a factor of 2.2 in the relative damage fields. By contrast, polycrystalline potassium chloride broke down at about the same intensity as its single-crystal form (about 8 GW cm⁻²). Thus, both types of silica seem to be very good laser materials but, what is more, a large improvement seems to be gained by virtue of the microscopic disorder of the amorphous form. The disorder in this material is on the scale of a few lattice constants, taking the form of a dispersion of bond angles in an otherwise continuous and compositionally ordered network. The polycrystalline potassium chloride, of course, is disordered but is made up of crystallites which are many hundreds of lattice constants in size. Thus, grain boundaries do not seem to interfere with the progress of the gross damage effects (fractures and so on), which are the indicators of when the

intrinsic damage fields are reached, but microscopic disorder apparently damps the breakdown effect quite drastically.

Although this is only a straw in the wind, a set of data for only one amorphous/crystalline pair, it is tempting to grasp at it and say, as the authors do, that the disordered network is more efficient at extracting energy from the electrons before they reach the energy necessary for impact ionisation: that is, it is more difficult to heat the electron distribution in the disordered material. This is, however, equivalent to saying that the electron mobility in amorphous silica at high fields is considerably lower than that for the crystalline form; this is unlikely, since Hughes has found that, for fields up to 10^7 V m⁻¹ (though impurity content caused some variation) the electron mobility and lifetime in synthetic fused silica was generally higher than for pure crystalline quartz. (See, for example, *Nature*, **246**, 190; 1973). Nevertheless, the study of high field breakdown in solids induced by a laser, when carried further, may well prove quite a versatile and useful method for investigating the transfer of energy from hot electrons to their solid host medium, with unique possibilities for the localisation and shortening of duration of the accelerating fields.

Singing muscles in a katydid

from our
Insect Physiology Correspondent

INSECTS are commonly regarded as cold blooded, or poikilothermic—their temperature agreeing with that of the environment. But it was well known to Newport, writing in Todd's *Cyclopaedia* in 1838, that the honeybee without visibly moving increases its body temperature well above that of its surroundings; and it is now known that this change is accompanied by action potentials in the thoracic muscles without vibration. It has been known since Girard in the middle of the last century that hawk moths increase their thoracic temperature by as much as 10° C by vibrating their wings. Such a rise in temperature increases the efficiency and frequency of wing muscle contraction. Recourse was had to this effect in trying to explain the very high rates of contraction and relaxation in the flight muscles of insects, which far exceed those of vertebrates. The rate of wing beat in the humming bird is 30–50 cycles per second: the blowfly will beat its wings at 150 cycles per second, a rate too rapid to be explained by a rise in temperature alone.

It was suggested that these wing muscles were operating in a state of incomplete tetanus. But the explanation

was ultimately found by Pringle who showed that these high rates of oscillation are myogenic: the rapid contraction of one set of muscles is induced by the stretching brought about by their antagonists; whereas shortening of the muscles leads to a rapid relaxation of tension. This same mechanism can explain the high rate of vibration in the timbal muscles of cicadas which may go up to 4,500 Hz.

It has now been shown by Josephson (*J. exp. Biol.*, **59**, 781; 1973) that in the singing katydids *Neoconocephalus* and *Eoconocephalus*, relatively high rates of sound pulses, rising to about 200 Hz, generated by rubbing the wings together, are controlled by synchronous, or neurogenic, muscles in which each contraction is initiated by a nerve impulse. The high rates of contraction are dependent on two factors: a rise in temperature to about 36° C (12° C above ambient temperature) in the thoracic muscles, and a consequent persistence of an unfused tetanus in the muscles at rates of 160 Hz or more.

This mechanism is expensive in energy, for each muscle must contract while its antagonist is still contracting, and the songs may continue for several hours on end. But the heat produced is necessary to maintain the required temperature; and the author suggests that the mechanism may have evolved as the result of sexual selection—the females opting for the males with the highest pulse frequencies in their song.

Stratigraphic record of Early Eocene *Hyopsodus* and the geometry of mammalian phylogeny

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Eocene strata in Wyoming spanning a period of 5 million years record a branching phylogeny which exhibits gradual phyletic evolution, overall size increase with iterative evolution of small species and character divergence following the origin of each new lineage.

THE gradual nature of evolutionary change was a central tenet in Darwin's *Origin of Species*¹. Darwin acknowledged that new forms of life appear suddenly in the fossil record and attributed this to gaps in the record. He stated that the stratigraphical column was probably not sufficiently complete to record in ancient lineages the continuous gradual change through natural selection that he and others had observed in domestic animals under artificial selection. The phylogeny of the condylarth *Hyopsodus* presented in this report is the first detailed record of mammalian evolution at the species level. The record is based on numerous superposed stratigraphical levels within one restricted geographical area of one geological formation: it provides new palaeontological evidence of Darwinian gradualism in evolution.

Phylogeny of *Hyopsodus*

Condylarths are archaic ungulates which are important because they were abundant in the early Tertiary and because the group includes the ancestors of the living Artiodactyla and Perissodactyla. During the past ten years, Yale expeditions have made large collections of fossil mammals from approximately 400 localities in the Early Eocene Willwood Formation^{2,3} of the Big Horn Basin in northwestern Wyoming. *Hyopsodus* is the most common mammal in these collections, being represented by approximately 4,000 jaw fragments containing two or more teeth. Morphologically, *Hyopsodus* had a long body with relatively short limbs (see inset in Fig. 1), suggesting a fossorial habit. In 1965 measurement of a stratigraphical section 1,690 feet (515 m) thick was completed by G. Meyer and L. Radinsky in the Antelope Creek-Elk Creek-Fifteen Mile Creek drainage area which covers a geographical area of approximately 20 km by 60 km, all of which is badlands with little or no cover of vegetation. The measured section spans most of Early Eocene time, a period of approximately 5 m.y.⁴.

The original stratigraphical section included forty-five localities yielding specimens of *Hyopsodus*, and the nearly horizontal attitude of strata in this area permitted interpolation into the original section of an additional ninety localities yielding *Hyopsodus* discovered since 1965. The stratigraphical position of each of these localities was determined to the nearest 20 feet (6.1 m), then the length and width of all lower first molars of *Hyopsodus* were measured and the log of the product of the measurements was computed for each successive interval. The product of length multiplied by width of M_1 is the best dental measurement for characterising the size of closely related mammalian species, and it is in general the best discriminant of closely related species occurring together in any one stratigraphical level⁵. Sympatric species of the same genus living today can only rarely be diagnosed by morpho-

logical differences in their cheek teeth, though the species usually differ significantly in size. Similarly, for the *Hyopsodus* from a given stratigraphical level, size is the only characteristic of the molars which permits species diagnosis.

Size is plotted against stratigraphical position in Fig. 1 for all specimens from the study area containing M_1 (a total of 928). In this plot a number of lineages are apparent, the best defined being those labelled *Hyopsodus latidens*-*H. minor*, *H. lysitensis* and *H. powellianus*. The range of variation of the sample of ninety-two *H. lysitensis* from level 1,360 is typical of living species, and undoubtedly closely approaches the maximum variation in size to be expected in any sample of a species of *Hyopsodus*. Samples at levels 440, 600, 660, and 1,240 have ranges of variation exceeding that expected of one species and the samples have tentatively been divided to indicate this, though they are not clearly bimodal.

The dashed lines in Fig. 1 indicate the limits of variation of each of the well defined lineages, and in addition show the most probable relationships of *H. simplex*, *H. wortmani*, *H. mentalis*, and *H. walcottianus* to the better represented species. It is apparent from the figure that the relatively small species *H. loomisi* gave rise in early Gray Bull time to two species *H. simplex* and *H. sp. A*. *Hyopsodus sp. A* gave rise in late Gray Bull time to *H. latidens* and *H. miticulus*. In the Lysite, *H. miticulus* gave rise to *H. lysitensis* and *H. powellianus*. *H. powellianus* apparently gave rise to *H. mentalis* and *H. walcottianus* at the beginning of Lost Cabin time. *H. minor* and *H. wortmani* are Lysite and Lost Cabin descendants of *H. latidens* and *H. lysitensis*, respectively.

The coexistence of three species of *Hyopsodus* in late Gray Bull, Lysite and Lost Cabin times is confirmed, although the relationships of each of these species are fundamentally different than has been proposed by recent authors. Gazin⁶ and Guthrie⁷, who grouped all specimens into either a lower Gray Bull, an upper Gray Bull, a Lysite or a Lost Cabin sample, proposed (using the species names of Fig. 1) that *H. loomisi* gave rise to a small, a medium and a large species, each of which then continued relatively unchanged through upper Gray Bull, Lysite and Lost Cabin strata. In other words they advocated a small lineage *H. simplex*-*H. minor*-*H. wortmani*, a lineage of medium-sized species *H. latidens*-*H. lysitensis*-*H. mentalis*, and a lineage of large species *H. miticulus*-*H. powellianus*-*H. walcottianus*. Division of the lower Eocene strata into forty-seven levels, instead of the four used by Guthrie and Gazin, shows that only the last group is a true evolutionary lineage. Whereas Gazin and Guthrie portray three lineages, each appearing suddenly and continuing almost unchanged through the remainder of the Early Eocene, it is clear from Fig. 1 that each new species evolved gradually from its parent species. Indeed, no species appeared abruptly, a fact which accords well with Darwin's view.

Each *Hyopsodus* lineage underwent gradual but significant size change. In the *H. latidens*-*H. minor* and *H. lysitensis*-*H. wortmani* lineages this change was sufficient to justify dividing each lineage into two time-successive phyletic species. Plotting the size data on a log scale makes the variability of samples of large and small species comparable but tends to

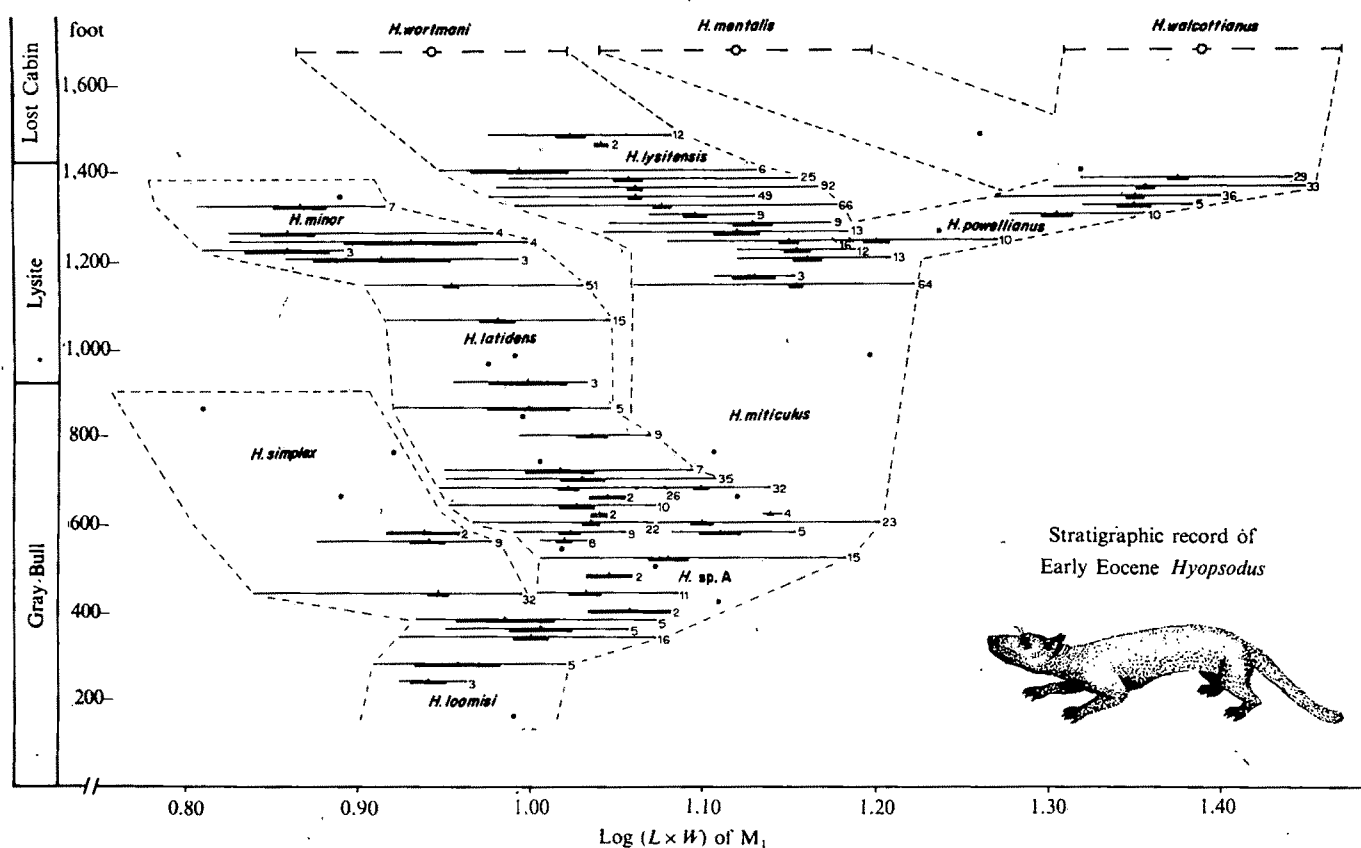


Fig. 1 Stratigraphical record and phylogeny of Early Eocene species of *Hyopsodus*. Abscissa is the \log_{10} of the length (mm) multiplied by width (mm) of the first lower molar. Ordinate is height (feet) above base of Willwood Formation, which includes Gray Bull, Lysite and Lost Cabin faunal zones. Sampling interval is 20 feet (6.1 m); average duration of one 20 foot interval is about 60,000 yr. Vertical slash is mean, heavy bar is standard error of mean, light bar is observed range, small numerals indicate sample size, and filled circles represent single specimens. Open circles and dashed lines at top of figure are means²⁸ and expected ranges for Lost Cabin species from the Wind River Basin which are poorly represented in the Willwood section. Inset, flesh restoration of *Hyopsodus* from Gazin⁶.

obscure the great overall increase in size in this radiation. The *Hyopsodus* radiation clearly conforms to Cope's rule, which states that there is a tendency for animal groups to evolve toward larger physical size (or from small size⁸). Of the eleven *Hyopsodus* species represented, however, six species became smaller and only five larger during the course of their evolution, indicating that Cope's rule does not deterministically describe evolution at the species level.

Iterative evolution, whereby a number of distinctive forms evolve repeatedly from one stem lineage, has been documented in the evolution of lobsters⁹ and in the evolution of pygidium morphology in Cambrian trilobites¹⁰. The same phenomenon is apparent in Fig. 1, where four small species *Hyopsodus simplex*, *H. minor*, *H. wortmani* and *H. mentalis* were derived successively and independently from the larger stem lineage *H. loomisi*—*H. sp. A*—*H. miticulus*—*H. powellianus*—*H. walcottianus*.

Palaeontological evidence such as that presented in Fig. 1 has occasionally been used to support the thesis that species arise sympatrically. In this case, as in earlier cases¹¹, allopatric speciation with subsequent migration may explain the fossil record equally well. It is clear from Fig. 1 that speciation must have preceded size divergence. Presumably speciation in *Hyopsodus* involved the evolution allopatrically of significant hybrid inviability between two populations, followed in sympatry by gradual size divergence to decrease interspecific competition¹².

The length of time observed before two lineages became clearly distinct in size is somewhat surprising. In the best documented case, *H. lysitensis* and *H. powellianus* seem to have required about 40 feet (12.2 m) of section, or on the order of 100,000 yr to become clearly differentiated. This slow, gradual divergence differs considerably from the expected rapid rate of displacement if resource competition between species was the

only controlling factor¹³. Species of *Hyopsodus* are the most common fossil mammals found in the Lower Eocene of North America. They presumably lived in populations of large size, and the slow rate of genetic change in large populations¹⁴ may have retarded the speed with which size displacement could be accomplished.

Geometry of mammalian phylogeny

A persistent gap in the palaeontological evidence for evolution has been the apparent absence in the fossil record of intermediate forms linking two species unequivocally to a common ancestor. Palaeontologists have generally reacted to this absence of intermediate forms by continued search for more complete stratigraphical sections. Recently a new 'punctuated equilibrium' model of animal phylogeny has been advanced^{15,16}, which explains the gaps in the fossil record as a natural result of allopatric speciation. According to this model, new species arise as peripheral isolates of a central population and the isolate, being small, has virtually no chance of being preserved in the fossil record. A successful isolate may replace the central population in a relatively short period of time—instantaneously considering the length of geological time. The postulated pattern of phylogeny resulting from this model includes long periods of time without significant morphological change in a lineage. Rarely this morphological equilibrium is punctuated by a rapid speciation event. The net result, according to the punctuated equilibrium model, is a fossil record of isolated static species without intermediate linking fossil populations.

The punctuated equilibrium model can be tested only by a relatively complete, stratigraphically controlled fossil record such as that of *Hyopsodus* already described. The phylogeny of *Hyopsodus* appeared^{6,7} to conform to the punctuated equi-

brum model and for this reason it was chosen to test the model.

Figure 1 shows clearly that when a large number of stratigraphical levels were studied within the Willwood Formation, the resulting phylogeny of *Hyopsodus* exhibited neither long periods of morphological equilibrium, nor rapid speciation events. Probably the chief reason why interspecies transitions are rare in the fossil record is, as Darwin proposed, because of the incomplete nature of most stratigraphical sections. Local stratigraphical successions that seem to be nearly complete, such as the Willwood Formation section, have still only rarely been studied in detail. In addition to the multi-lineage radiation described in this report, a number of other examples in the literature exhibit phyletic gradualism in single lineages of invertebrates¹⁷⁻²⁰ (although details of these studies have been challenged, for numerous characters gradual evolution remains the most parsimonious interpretation, given the fossil record in each case) and vertebrates²¹⁻²⁵. The punctuated equilibrium model remains to be substantiated by a detailed stratigraphical study within a single geological formation.

The fossil record of *Hyopsodus*, when studied in detail, seems to justify Darwin's view that species evolved gradually. Study of other characters in other phylogenies of species with a good fossil record is needed to determine the extent to which population size, character variability²⁶ and other factors affect the rate of evolutionary divergence following speciation.

The stratigraphical record of human evolution unfortunately still includes many gaps; study of the available evidence in a stratigraphic context²⁷, however, suggests that hominid phylogeny conforms to the pattern of gradual-character divergence exhibited here by *Hyopsodus*.

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Photoreactivating enzyme from human leukocytes

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A photoreactivating enzyme has been found in human leukocytes. The specificity of the enzyme for cyclobutyl pyrimidine dimers in DNA may allow direct evaluation of the role of dimers in ultraviolet-induced carcinogenesis in man.

PHOTOREACTIVATION is the specific repair of ultraviolet (220-300 nm)-induced cyclobutyl pyrimidine dimers in DNA¹. The reaction is carried out by the photoreactivating enzyme, which requires light in the range 300-500 nm for its activity^{2,3}. The specificity of the enzyme for pyrimidine dimers enables its use as a diagnostic tool: if ultraviolet-induced biological damage is photoreactivable, pyrimidine dimers are probably a major cause of the damage⁴. This approach has been used to show that dimers are largely responsible for ultraviolet-induced death and mutation in prokaryotes and simple eukaryotes⁵⁻⁷.

Ultraviolet light produces skin cancer in humans, which in the extreme case of xeroderma pigmentosum leads to death⁸. Consequently, it is important to evaluate directly the role of dimers in the induction of such cancers in man. The apparent lack of the photoreactivating enzyme in placental

mammals^{4,9} has prevented direct evaluation of the role of dimers in human carcinogenesis although studies with model systems are in progress¹⁰.

I show here that human cells do have a photoreactivating enzyme. I have purified the enzyme to apparent homogeneity and have studied some of its physicochemical properties.

Radioactive DNAs were prepared as previously described¹¹. The specific activity of the ³²P-labelled T7 DNA was 397 c.p.m. pmol⁻¹ and that of the ³H-thymidine-labelled T7 DNA was 12.5 c.p.m. pmol⁻¹. Pyrimidine dimers were produced by exposure to 254 nm radiation; dimer content of ³H-thymidine-labelled DNA was determined by formic acid hydrolysis, chromatography in *n*-butanol: acetic acid: H₂O (40:6:15), elution and counting of dimer-containing and monomer-containing regions of the chromatogram in a scintillation counter¹². For the ³²P-DNA, the dimer content was determined by (1) digestion of the DNA with DNase I, venom phosphodiesterase and alkaline phosphatase to a mixture of inorganic phosphate, mononucleosides and dimer-containing ³²P-labelled oligonucleotides; (2) adsorption of the labelled oligonucleotides to Norit, and (3) counting in a planchet counter¹¹.

TABLE 1 Summary of purification procedures

Procedure	Fraction	Volume (ml)	Protein (mg ml ⁻¹)	Units (pmol h ⁻¹)	Specific activity (pmol mg ⁻¹ h ⁻¹)
Cell extract	1	2.0	82	400	2.44
Ammonium sulphate pellet	2	1.0	6.2	256	41.4
Isoelectric focusing pool	3	1.0	0.015	95	6,350.0

Photoreactivating enzyme activity was assayed by adding cell extract or purified enzyme to 0.2 ml 0.02 M potassium phosphate buffer, pH 7.2, containing 0.1 mM dithiothreitol, 0.1 mM EDTA and 30-100 pmol of ultraviolet irradiated, ³²P-labelled T7 DNA. For each determination one assay tube was placed in the dark and another was exposed to photoreactivating light from a General Electric 150-W spot lamp. The dimer content of the samples was then determined as described above. Photoreactivating activity is the difference in dimer content of the light and dark samples; units of photoreactivation are pmol⁻¹ mg⁻¹ h⁻¹. Protein concentrations were determined by the Lowry method¹⁸, using bovine serum albumin as the protein standard.

The enzyme

Plasmagel (Bellon Labs) (4 ml) was added to 12 ml of freshly drawn human peripheral blood treated with 170 U of heparin. The suspension was mixed gently and allowed to settle 45 min at 37°C. The supernatant suspension, from which most of the erythrocytes had settled, was centrifuged at 2,000 r.p.m. in the SS-34 rotor of the Sorvall RCB-2. The cells were washed three times by gentle resuspension in 2 mM potassium phosphate buffer, pH 7.2, containing 0.15 M NaCl and centrifugation as before. Washing removed serum proteins and proteins added with the Plasmagel. The cells

TABLE 2 Characterisation of the photoreactivating enzyme activity

Enzyme	Ultraviolet exposure to ³² P-labelled T7 DNA (erg mm ⁻²)	Photo-reactivating light exposure (min)	Photo-reactivating enzyme activity (pmol h ⁻¹)
Fraction 3	3,000	0	0.1
Fraction 3	3,000	30	10.3
Fraction 3, trypsin treated, 34°C 5 min	3,000	30	6.53
Fraction 3	0	30	0.12
None	3,000	30	0.02

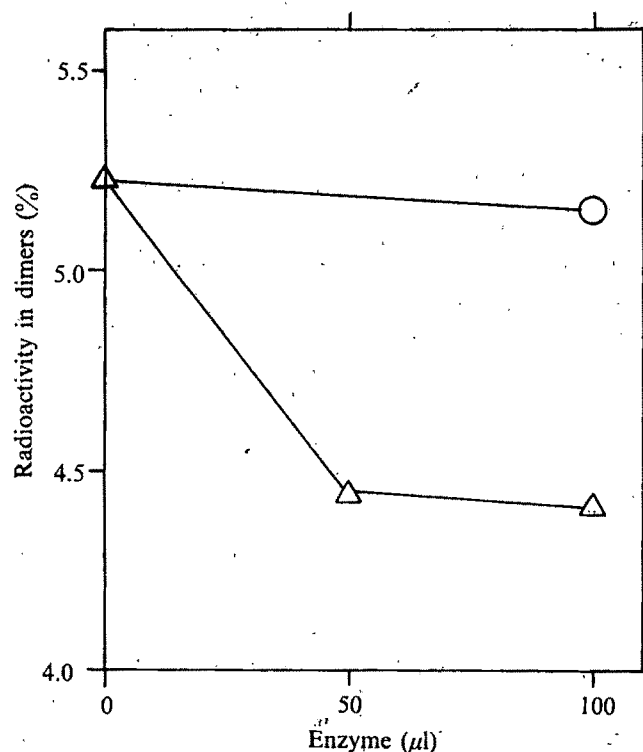


Fig. 1 Photoreactivation of dimers in ³H-thymidine-labelled DNA. Fraction 2 enzyme was added to 155 nmol of dimer-containing ³H-thymidine-labelled DNA in a standard assay mixture. After 5 min incubation at 37°C in the dark, one sample (○) was kept in the dark while the others (Δ) were exposed to 30 min of photoreactivating light. The dimer content of the samples was determined by the method of Setlow *et al.*¹².

were suspended in 2 ml of buffer E (10 mM Tris, pH 7.0, 0.1 mM EDTA, 0.1 mM dithiothreitol) and sonicated for 45 s in a Kontes sonicator. The resulting solution was fraction 1. Solid ammonium sulphate (0.163 g ml⁻¹ of fraction 1) was added and the mixture was stirred gently for 15 min on ice. The solution was centrifuged for 15 min at 15,000 r.p.m. in the SE 12 rotor of the Sorvall. Less than 30% of the photoreactivating enzyme activity appeared in the pellet; to the supernatant was added 0.163 g (per ml of fraction 1) of ammonium sulphate; the mixture was stirred 15 min after all the ammonium sulphate had dissolved, and centrifuged in the SE12 rotor as before. All the enzyme activity appeared in the pellet, which was dissolved in 1 ml buffer E (fraction 2). Fraction 2 was layered into the middle of an isoelectric focusing gradient (110 ml) prepared as follows: the cathode was placed at the bottom of the column. The cathode solution contained 14 ml H₂O, 0.2g NaOH and 12g sucrose. Sample of 55 ml of a 110 ml linear density gradient (formed by mixing in a LKB gradient apparatus a solution of 47.8 ml glycerol, 5 μl 0.1 M EDTA, 5 μl 0.1 M dithiothreitol, 9.83 ml H₂O and 1.88 ml LKB pH 3.5-10 ampholine solution with a solution of 57.4 ml H₂O, 5 μl 0.1 M dithiothreitol and 0.625 ml of the ampholine solution) was layered into a 110 ml jacketed isoelectric focusing column maintained at 4°C by a Lauda circulator. Fraction 2 was layered onto the gradient solution, and the rest of the gradient solution was then added. The anode solution (0.1 ml phosphoric acid, 10 ml H₂O) was layered on top of the gradient. A voltage of 300 V was applied across the column for 72 h. Fractions (0.5 ml) were collected and assayed for photoreactivating enzyme activity. Although several small peaks of enzyme were detected at pH values greater than 5.4, most of the enzyme was located at pH 5.4. The peak fractions were combined to yield fraction 3. The purification procedure is summarised in Table 1.

As Table 2 shows, the purified enzyme requires ultraviolet-irradiated DNA and photoreactivating light for its activity. The enzyme preparation must be added to the DNA for the reaction to proceed. The most important property of the enzyme is its ability to monomerise pyrimidine dimers in DNA. Since the ³²P-DNA assay for photoreactivation is indirect, I tested the ability of the enzyme preparation to reverse dimers in ³H-thymidine-labelled DNA. In this case both dimers, TT and CT, and the monomer, T, can be isolated and measured directly. Figure 1 shows that in the presence of photoreactivating light the enzyme preparation causes dimers to disappear from the ³H-thymidine-labelled DNA. In the dark the dimer content decreases very little, if at all; thus

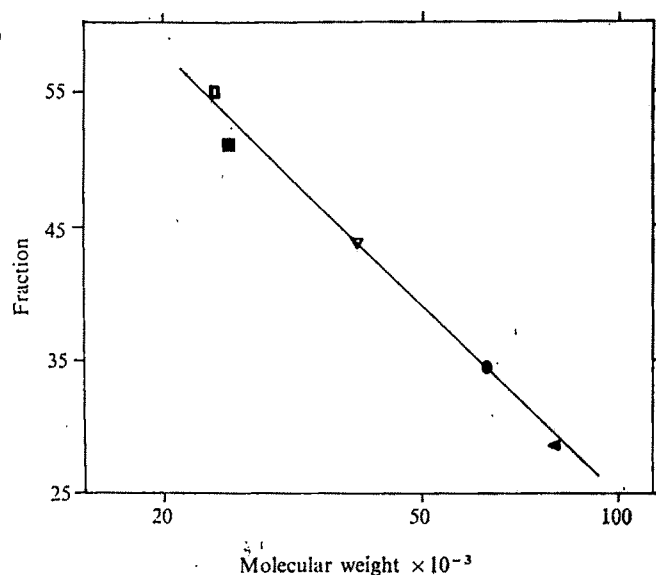


Fig. 2 Molecular weight determination. The elution position on a 27×1.1 cm column of Biogel P-100 in buffer E + 1.0M NaCl of protein standards (bacterial alkaline phosphatase (▲), horse haemoglobin (●), cytochrome c dimer (■) and trypsin (□) was determined. The elution position of the human photoreactivating enzyme (V) was used to estimate its molecular weight at 40,000.

the disappearance of dimers from DNA in the light is due to true monomerisation rather than excision. The demonstration of photoreactivation by the 32 P-DNA assay also indicates that excision did not occur, as this assay does not distinguish excised dimers (in short pieces of DNA) from those remaining in high-molecular-weight DNA¹¹.

Since several types of low molecular weight molecules sensitise monomerisation of dimers¹⁴⁻¹⁶, it was important to show that the species in this preparation responsible for dimer monomerisation has a protein component. Treatment of the enzyme with 10 mg trypsin for 1 and 5 min at 34° C decreased enzyme activity to 85 and 59% of its original value, respectively. In this time the treatment at 34° C without trypsin did not affect enzyme activity. It is thus likely that the molecule responsible for photoreactivation has a protein component.

The enzyme has an apparent molecular weight of 40,000 (Fig. 2). The isoelectric pH of the enzyme is 5.4, which agrees qualitatively with the rapid migration towards the anode of the enzyme on polyacrylamide gels carried out under non-denaturing conditions (see below).

As with the *E. coli* photoreactivating enzyme, the pH optimum of the human enzyme is 7.2. At pH values 8.1 and 6.8 the enzyme showed 29 and 49% of the activity exhibited at pH 7.2. However, the human enzyme is inhibited by high ionic strength and at $\mu = 0.18$, the optimum for the *E. coli* enzyme, the human enzyme has only 20% of the activity it has at $\mu = 0.05$.

The final enzyme preparation, fraction 3, yields one major protein band on electrophoresis under non-denaturing conditions (Fig. 3). That this band is photoreactivating enzyme was shown by slicing a gel into 0.5 cm lengths, eluting the protein by electrophoresis from the gel and assaying each fraction for photoreactivating enzyme activity. The major band of enzyme activity coincided with the major protein band on the gel. In addition, two smaller peaks of enzyme activity appeared in the regions 0-1 and 0.3-0.4 of the gel. Since fraction 3 enzyme is isolated from a narrow band of the isoelectric focusing column, it is unlikely that these smaller peaks represent multiple enzyme species; rather they are probably aggregates of the enzyme into dimer and higher multiples. Such aggregates have also appeared in glycerol gradients performed in conditions of low ionic strength.

Photoreactivation in mammalian cells

Although photoreactivation is found in all phyla and in many types of cells⁴, data on the presence of the photoreactivating enzyme in placental mammals have been contradictory¹⁷⁻²². The problems in interpreting these data are, (1) for positive results, to distinguish between true cases of enzymatic photoreactivation and effects due to photoprotection, and (2) for negative results, to differentiate true absence of the enzyme from inadequate exposure or insensitive assay procedures. Clearly photoreactivation is more difficult to demonstrate *in vivo* in mammalian cells than in other cells; whether this is due to the low cell content of the enzyme or to the inaccessibility of the DNA to the enzyme is not clear. Cook⁴ reported an example of the latter: cell cultures of the South American woolly possum (*Caluromys derbianus*) Tasmanian potoroo (*Potorus tridactylis*) and opossum (*Didelphis marsupialis*) (all marsupials) had detectable photoreactivating enzyme when assayed *in vitro* whereas *in vivo*, they required very long exposures (6 h) to photoreactivate 40% (potoroo) and 65% (woolly possum) of their dimers. But if inaccessibility of DNA had been the cause, Cook and McGrath⁹ should have detected enzyme activity in placental mammals *in vitro*. These are two possible explanations for their failure to do so. First, although they assayed various tissues, they did not test mammalian leukocytes, in spite of the high activity they found in toad leukocytes. Second, they may not have been able to detect the enzyme if it had been present. Unlike the yeast and *E. coli* enzymes, the human enzyme is inhibited at high ionic strength, and at

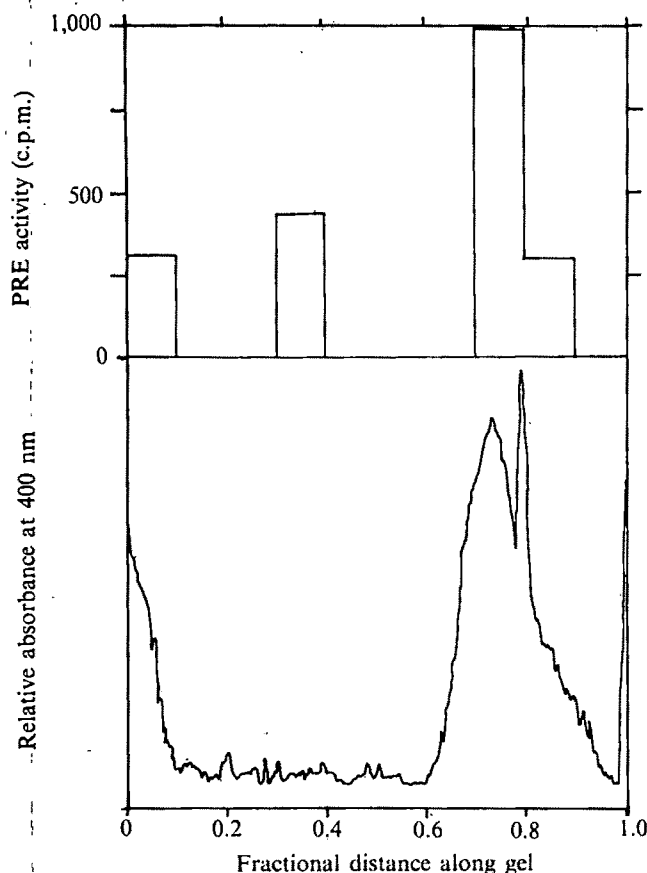


Fig. 3 Absorbance and photoreactivating activity profiles of fraction 3 enzyme. The 5% gels were prepared according to the method of Davis²³. Two gels were prepared: after electrophoresis, one was stained in Coomassie blue and, after destaining, was scanned in a Gilson spectrophotometer for determination of protein distribution. The other gel was sliced into 0.5 cm segments; the enzyme was eluted by electrophoresis and assayed for photoreactivating enzyme activity as usual. The peak at 0.81 is tracking dye.

the ionic strength used by Cook and McGrath (0.11) it would have been only 35% as active as under optimal conditions ($\mu = 0.05$).

Photoreactivation is of primary biological interest as an analytical tool. Since the photoreactivating enzyme acts specifically on cyclobutane-type pyrimidine dimers, true enzymatic photoreactivation of ultraviolet-induced biological effect indicates that pyrimidine dimers were the molecular alteration responsible for the damage. Such tests have shown that dimers are a primary cause of death and mutation in prokaryotic and eukaryotic cells⁵⁻⁷.

Since sunlight induces cancer in man, it is tempting to speculate that pyrimidine dimers are the molecular cause of such cancers. But so far, it has not been possible to test this hypothesis directly and model systems have been necessary: Hart and Setlow presented data indicating that ultraviolet-induced, potentially tumorigenic lesions in the fish *Poecilia formosa* can be photoreactivated¹⁰. But now it should be possible to select a human or other placental mammalian cell type and photoreactivation conditions optimal for the direct evaluation of the role of pyrimidine dimers in the induction of cancerous growth by ultraviolet light.

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Specialised transformation in *Escherichia coli* K12

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A specialised transformation system has been developed in E. coli K12 by using specialised transducing phage DNA as donor DNA. Transformation of a marker on a F' episome has also been achieved.

GENERALISED transformation for chromosomal markers in *Escherichia coli* K12 has been reported recently¹⁻⁴. These studies demonstrated two major factors in determining the transformability of *E. coli*: CaCl_2 treatment of the cell surface to allow permeation of DNA and an appropriate intracellular status of various DNases, especially the ATP-dependent (*recB-recC*) DNase⁵⁻⁸. In this, as in other bacterial generalised transformation systems, the chromosomal donor DNA which is used is heterogeneous with respect to molecular size and genetic information. A transformation system in which essentially all the donor DNA is homogeneous in its genetic composition and its molecular size would be advantageous for studying various aspects of transformation, especially for studying molecular mechanisms of recombination. DNA from specialised transducing *E. coli* phages seems to be the ideal source of transforming donor DNA for this purpose because of the well characterised DNA structure and the relative ease of obtaining a large quantity of pure DNA which is unique for a specific region of the chromosome.

We report here such specialised transformation of a recipient chromosomal as well as an episomal (*F'*) genetic marker. This was demonstrated by using donor DNA isolated from phages $\phi 80\text{pt}^{9,10}$ and λpt^{11} , which carry tryptophan genes, to transform a tryptophan-requiring strain to tryptophan prototrophy.

Isolation of transforming DNA from *E. coli* (Hfr C6) was described previously⁴. The phage DNA was isolated by a phenol procedure¹² after purifying the phages by CsCl density centrifugation. The DNA preparations were extensively dialysed against 1/10 SSC (15 mM NaCl — 1.5 mM Na-citrate), and were heated at 70° C for 10 min before use. Shearing of the DNA was carried out with a Sorvall Omni mixer. Transformation was performed as described previously⁴, except that the reaction was stopped by the addition of an equal volume of cold 50 mM sodium citrate. Bacterial and phage strains and all the mutant markers are described in Table 1. In the text only the relevant mutations of each strain will be indicated.

Specialised transformation

Tables 2 and 3 show typical results of experiments involving specialised transformation of a *trp* mutation (*trpB*) with $\phi 80\text{pt}$ (*trpA*⁺*B*⁺*C*⁺) and λpt (*trpA*⁺*B*⁺*C*⁺*D*⁺*E*⁺) DNA. The

TABLE 1 Bacterial and phage strains used in this study

(A) <i>E. coli</i> K12 strains	Derived from	Relevant genetic characteristics used in this study*	Source
M0639	JC7623	<i>recB21 recC22 sbcB15 leu-6 trpB9579</i>	This laboratory†
M0671	M0639	<i>recB21 recC22 sbcB15 leu-6 trpB9579 tonB</i>	This laboratory†
M0668	M0639	<i>recB21 recC22 sbcB+ leu-6 trpB9579</i>	This laboratory§
M0649	M0639	<i>recB+ recC+ sbcB15 leu-6 trpB9579</i>	This laboratory§
M0650	M0639	<i>recB+ recC+ sbcB+ leu-6 trpB9579</i>	This laboratory§
M0640	JC7623	<i>recB21 recC22 sbcB15 leu-6 ΔtonBtrpABCDE</i>	This laboratory
M0646	M0640	<i>F'(trpB9579 tonB+ attφ80+)/M0640</i>	This laboratory
M0868	CH56	<i>ΔtonBtrpABCDE recA1</i>	This laboratory**
(B) Bacteriophage strains	Strains		Source
	φ80pt(<i>trpA+B+C+</i>)		H. Ozeki
	φ80pt(<i>trpA+B-C+</i>)		This laboratory††
	φ80pt(<i>trpD+E+</i>)		F. Imamoto
	φ80		F. Imamoto
	λpt(60-3 <i>trpA+B+C+D+E+O+</i>)		F. Imamoto
	λel26		R. J. Huskey

* Besides the genetic characteristics listed here, M0639, M0671, M0649 and M0640 have the following markers: *his-4 ara-14 thr-1 thi-1 lacY-1 ml-1 xyl-6 galK2 proA2 argE3 str-31 tsx-33 sup-37* amber. In addition to the above markers, M0650 is *his+* and M0668 is *his+ thy-*.

† Constructed by transformation with DNA from W3110 (*trpB9579*)¹² provided by F. Imamoto.

‡ *tonB* mutation introduced by mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine.

§ Constructed by conjugation with Hfr KL16.

|| Constructed by transformation with DNA from strain CH56 provided by W. Maas.

** Original F' is described in ref. 14. Before transfer to M0640, the *trpB9579* mutation was introduced and the *lac* genes were deleted.

†† Constructed by mating with Hfr KL16-99.

‡‡ Isolated from phages released from a φ80pt(*trpA+B+C+*) lysogen of W3110 (*trpB9579*)¹³.

φ80pt (*trpA+B+C+*) used as donor DNA carries a part (*trpABC*) of the *trp* operon of the *E. coli* chromosome, in place of the *int-red* region of the φ80 genome. Therefore, the φ80pt is *int- red-* and *trpA+B+C+* (ref. 15). This *int- red-* condition of φ80pt eliminates the phage recombination systems which might have complicated studies of the host recombination system. For similar reasons, we have also used DNA from λpt(*trpA+B+C+D+E+*), a plaque-forming phage λ containing the entire *trp* operon of *E. coli* in place of at least the *int-red* region of the bacteriophage λ chromosome as in φ80pt. As Table 2 shows, when CaCl₂ treated cells of the multiauxotrophic transformable *E. coli* K12 strain (M0671 *recB21 recC22 sbcB15 trpB9579 leu-6 tonB*) were exposed to unshredded, intact DNA isolated from φ80pt(*trpA+B+C+*), considerable numbers of Trp⁺ transformants appeared on the selective plates, but no Leu⁺ transformants were detected. The same degree of specialised transformation for *trpB* was also observed with sheared φ80pt(*A+B+C+*) DNA which had an average molecular weight of 8×10^6 , approximately a quarter the size of a φ80 genome (31×10^6)¹⁶. Fig. 1 shows the profile of transforming activities of the sheared φ80pt DNA after sucrose gradient centrifugation, showing that the transforming activity observed with the sheared DNA is not due to transformation by any residual intact φ80pt DNA

TABLE 2 Transformation of the *trpB* mutation with φ80pt and λpt DNA

Source of DNA	No. of transformants	
	Trp ⁺	Leu ⁺
None	0	0
φ80pt (<i>trpA+B+C+</i>)	1,808	0
φ80pt (<i>trpA+B+C+</i>), sheared	1,814	2
λpt (<i>trpA+B+C+D+E+</i>)	1,158	0
λpt (<i>trpA+B+C+D+E+</i>), sheared	840	0
<i>E. coli</i> Hfr C6 (<i>trp+ leu+</i>), sheared	106	144

This transformation was performed as described previously⁴ with a slight modification (see text) using M0671 as the recipient cells. The concentrations of the DNA used were 5 μg per 0.5 ml for φpt and λpt DNA and 10 μg per 0.5 ml for *E. coli* DNA. The average molecular weight of sheared DNA was 8.5×10^6 , 8.9×10^6 and 10×10^6 for φ80pt, λpt and *E. coli* DNA, respectively. The number of transformants is presented as the sum of the Trp⁺ or Leu⁺ colonies produced with a total of 2.2×10^7 viable recipient cells.

molecules. A slight shift of the activity from the peak of the absorbance profile of the quarter molecules toward the higher molecular weight side is probably due to the unique effect of the molecular weight of donor DNA in the transformation of *E. coli*; maximum transforming activity was

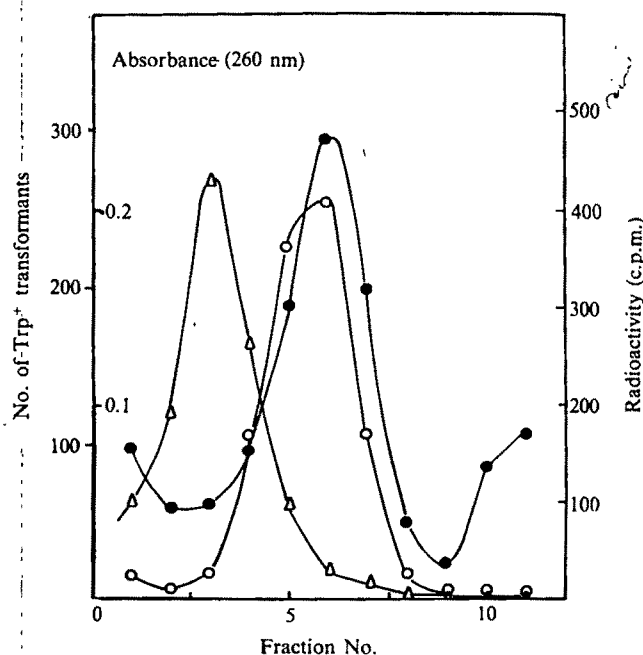


Fig. 1 Sucrose gradient centrifugation of sheared φ80pt DNA. Fifty μg of sheared φ80pt(*A+B+C+*) and ³H-φ80 DNA (0.05 μg, reference marker) in 0.5 ml of 1/10 SSC were applied to an 11.5 ml gradient of 5–20% sucrose in a buffer of 20 mM Tris-HCl, pH 7.6, 1 M NaCl and 1 mM EDTA. The centrifugation was performed at 36,000 r.p.m. for 5 h at 20°C using a Spinco SW41 rotor. Fractions (1 ml) were collected, a part (0.2 ml) of each fraction was withdrawn for radioactivity assays and the rest was dialysed against 1/10 SSC. Transformation was performed as described previously⁴ using 0.1 ml of the dialysed fractions as donor DNA and M0671 as a recipient. The number of transformants is the sum of Trp⁺ colonies produced with a total of 8×10^6 recipient cells. Δ, ³H-φ80 DNA; ○, transformants; ●, A_{260 nm}.

observed with DNA the molecular weight of which was between 1×10^6 and 2×10^6 (refs 3 and 4). Essentially the same results were obtained with unsheared and sheared λ pt(*trpA*⁺*B*⁺*C*⁺*D*⁺*E*⁺) DNA (Table 2).

Table 3 shows that the transforming activity increased linearly up to 5 μ g DNA per tube (0.5 ml) then levelled off, more or less the same pattern as observed when chromosomal DNA was used. The maximum transformation frequency with these DNA preparations was 2×10^{-5} to 8×10^{-5} . This is ten to thirty times greater than that observed with *E. coli* chromosomal DNA (1×10^6 to 5×10^6)¹⁻⁴. This higher efficiency is to be expected, since the concentration of the *trpB* gene in the ϕ 80pt DNA (also λ pt DNA) preparation was approximately eighty times greater than that in the chromosomal DNA preparation. The observed lower transformation efficiency than expected (eighty-fold increase) may have been due to recipient base sequence non-homology with the phage-specific base sequences which lie adjacent to the *trp* genes in the donor DNA. The biologically active material in the DNA preparation was sensitive to DNase (Table 3), eliminating the possibility that transduction by contaminating intact phages was the source of transformants. A ϕ 80 resistant (*tonB*) mutant strain was used as recipient for most experiments as a precaution to exclude such a possibility.

As Table 4 shows, the DNA from other types of ϕ 80 and λ phages, ϕ 80pt(*trpA*⁺*B*⁺*C*⁺), ϕ 80pt(*trpD*⁺*E*⁺), wild type ϕ 80 and λ , did not give any Trp⁺ transformants, clearly indicating that the presence of the *trpB*⁺ gene in the donor DNA preparation is the source of the transformation of the recipient's *trpB*⁻ marker. We next investigated the effect on specialised transformation of the genetic background of the recipient cells with respect to *recB* *recC* (ATP-dependent DNase) and *sbcB* (exonuclease I)¹⁷ genes, because generalised transformation of *E. coli* was shown to be affected by these genes¹. As we found in that system, the highest specialised transformation efficiency by ϕ 80pt(*A*⁺*B*⁺*C*⁺) DNA was observed in recipient strain M0639 (*recB21 recC22 sbcB15*) in which both DNases were missing. A small but significant number of transformants, approximately 20% of that observed with M0639, was obtained with M0649 (*recB*⁺ *recC*⁺ *sbcB15*).

TABLE 3 Transformation of the *trpB* mutation with various concentrations of DNA

DNA	Concentration (μ g per 0.5 ml)	No. of Trp ⁺ transformants
None	0	0
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺)	1	140
	2	213
	5	458
	10	544
	10 (+DNase)	1
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺), sheared	1	334
	2	557
	5	1,090
	10	1,017
	10 (+DNase)	2
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺)	1	171
	2	167
	5	273
	10	603
	10 (+DNase)	2
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺), sheared	1	180
	2	231
	5	700
	10	714
	10 (+DNase)	1

Transformation was performed as described previously⁴ with a slight modification (see text) using M0671 as the recipient cells. The average molecular weight of sheared DNA was 8.5×10^6 and 8.9×10^6 for ϕ 80pt and λ pt DNA, respectively. DNase was added at a concentration of 10 μ g per tube (0.5 ml). The number of transformants is presented as the sum of the number of Trp⁺ colonies produced with a total of 7×10^7 viable recipient cells.

TABLE 4 Transformation of the *trpB* mutation with DNA from various sources

Source of DNA (genotype)	No. of transformants
None	0
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺)	1012
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺), sheared	1333
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺)	1
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺), sheared	1
ϕ 80pt(<i>trpD</i> ⁺ <i>E</i> ⁺)	0
ϕ 80pt(<i>trpD</i> ⁺ <i>E</i> ⁺), sheared	0
ϕ 80	0
ϕ 80, sheared	0
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺)	648
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺), sheared	770
λ (cI26)	0
λ (cI26), sheared	3

Transformation was performed as described previously⁴ with a slight modification (see text) using M0671 as the recipient cells and DNA at a concentration of 5 μ g per tube (0.5 ml). The average molecular weight of sheared DNA from various phages was in the range of 8×10^6 to 9×10^6 . The number of transformants is the sum of Trp⁺ colonies produced with a total of 1.9×10^7 viable recipient cells.

Transformation occurred to a much lesser extent, approximately 5% of that with M0639, with B0650 (*recB*⁺ *recC*⁺ *sbcB*⁺). No transformants were obtained with M0668 (*recB21 recC22 sbcB*⁺), which is recombination-deficient. The absence of transformation in the Rec⁻ recipient indicates the participation of the host recombination system in the transformation process.

Mechanism of transformation

We have characterised the nature of the transformants obtained in our specialised transformation system. All the 120 tested Trp⁺ transformants obtained with sheared DNA were sensitive to ϕ 80 and did not produce any phage particles even after ultraviolet irradiation. Almost all (117 out of 120) the Trp⁺ transformants obtained with unsheared DNA were also sensitive to ϕ 80 and unable to produce phage after irradiation with ultraviolet light. These ϕ 80-sensitive Trp⁺ transformants produced by sheared and unsheared DNA were stable in their acquired Trp⁺ character. We could not detect any Trp⁻ segregants among more than 5,000 progeny grown from representative Trp⁺ colonies in a non-selective medium. We also tried to determine whether some Trp⁺ transformants still possessed the original *trp*⁻ markers in addition to the newly acquired Trp⁺ character. The *trp* operon and the *cysB* gene are known to be about 50% linked by cotransduction with P1 phage. The studies on specialised transduction had shown that integration events could place the newly added *trp*⁺ marker sometimes proximal and sometimes distal to the *trp*⁻ marker with respect to *cysB* (ref. 15). In the latter case, at least, it would be possible by P1 transduction readily to transfer the *trp*⁻ marker linked to *cysB* and without the *trp*⁺ marker. Twenty P1 lysates were prepared from random ϕ 80-sensitive Trp⁺ transformants (ten each from Trp⁺ transformants obtained with sheared and non-sheared DNA) and transduction was performed with a *trp*⁺ *cysB*⁻ recipient. All Cys⁺ transductants (more than 5000 Cys⁺ were tested) were Trp⁺, suggesting that the pre-existing *trp*⁻ marker was not present and had been replaced by a *trp*⁺ marker in all twenty transformants. These results, plus the fact that the transformation can be accomplished with sheared DNA suggest that the mechanism of transformation of the *trp* marker does not involve addition of the entire ϕ 80pt genome to the *E. coli* chromosome, but rather the replacement of the pre-existing *trp*⁻ marker by the *trp*⁺ marker present in the ϕ 80pt donor DNA. This is similar to what occurs in generalised transformation in *E. coli* and is in sharp contrast to specialised transduction

TABLE 5 Transformation of the *trpB* mutation on F' episome

Strain	Relevant genotype	Source	DNA	Concentration (μg per 0.5 ml)	Transformation frequency (trp^+ per 10^6 cells)
M0646	<i>F'lonB⁺trpB⁻/ΔtonBtrpABCDE</i>	None $\phi 80\text{pt}(\text{trpA}^+\text{B}^+\text{C}^+)$		0	<0.1
				1	7.4
				2	18.1
				5	56.7
				10	51.1
				10 (+DNase)	<0.1
		$\phi 80\text{pt}(\text{trpA}^+\text{B}^+\text{C}^+)$, sheared		1	20.3
				2	30.7
				5	63.7
				10	29.3
				10 (+DNase)	<0.1
				10	73.3
M0640	<i>ΔtonBtrpABCDE</i>	None $\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$		10 (+DNase)	<0.1
				10	78.1
				10 (+DNase)	0.4
				10	<0.1
				10 (+DNase)	<0.1
				10	<0.1
		$\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$, sheared		10	<0.1
				10	<0.1
				10	<0.1
				10	<0.1

Transformation was performed as described previously⁴ with a slight modification (see text). Because of the temperature sensitivity of the F' replication, the cells (M0640 and M0646) were grown at 35°C and the selective plates were incubated at 30°C after transformation. The molecular weights of sheared DNA used here were 8.5×10^6 and 8.9×10^6 for $\phi 80\text{pt}$ and λpt DNA, respectively. DNase was added at a concentration of 10 μg per tube (0.5 ml). Trp^+ transformants were scored and the transformation frequency was calculated according to the total number of 9.4×10^6 and 2.7×10^7 recipient cells produced by M0640 and M0646, respectively.

with $\phi 80\text{pt}$ ¹⁵ and to transformation with λdgal DNA¹⁸, in which the entire $\phi 80\text{pt}$ and λdgal phage genomes are integrated into the host chromosome.

Transformation of *trp*⁻ on F' DNA

We have also accomplished specialised transformation of a *trpB* mutation located on episomal F' DNA instead of on the chromosome. A transformable strain (M0646) was constructed harbouring an F' which carried the *trp* operon (with a *trpB9579* mutation) plus adjacent chromosomal regions including the *tonB* locus, and with the *trp* operon and *tonB* locus deleted from the chromosome. When this strain was used as recipient for $\phi 80\text{pt}(\text{A}^+\text{B}^+\text{C}^+)$ and $\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$ donor DNA, Trp^+ transformants emerged (Table 5). The frequency of the transformation (5×10^{-6} – 10×10^{-6}) was somewhat lower than the frequency obtained with the same donor DNA but with the recipient's *trpB9579* allele located on the chromosome. Since the F'-bearing recipient cells (M0646) contained a large deletion on the chromosome, from the *tonB* locus through the entire *trp* operon, the Trp^+ transformants seemed likely to be the products of recombination between the homologous *trp* regions on the F'DNA and the $\phi 80\text{pt}$ donor DNA. This was confirmed by our findings that (1) no Trp^+ transformants were obtained when we use recipients (M0640) harbouring the same large chromosomal deletion but lacking the episome (Table 5), and (2) the Trp^+ character could be transferred from Trp^+ transformants to a recombination deficient F' strain (M0868 *recA1 ΔtonBtrpABCDE*) together with other characters associated with the F', such as normal chromium resistance determined by the *tonB* locus^{19,20}. The basic characteristics of the system (Table 5) seem to be the same as those obtained with specialised transformation of the chromosomal *trp* marker.

Value of system

We have described, the transformation of a genetic marker (*trp*) on the *E. coli* chromosome or an F' episome by DNA isolated from specialised transducing phages carrying the *trp*⁺ marker. Although the frequency of the transformation ($\sim 10^{-5}$) is still somewhat lower than that obtained in generalised transformation with other bacterial species, for

example *Bacillus subtilis*, this system provides certain special advantages for studying various aspects of the transfer of genetic information, especially the mechanism of genetic recombination. The homogeneity of the genetic and molecular structure of donor DNA, stemming from its specialised transducing phage origin, is unique for bacterial transformation systems. The extensive genetic and biochemical background knowledge of *E. coli* and its related phages will be another advantage to this system. Of course, specialised transformation is not limited to *trp* genes but should also be applicable to any other well-characterised genes in *E. coli* such as *gal* or *bio* genes whose specialised transduction by λ phage is well established. Transformation of F' DNA with phage DNA reported here will provide a system in which not only the donor DNA but also the recipient DNA is composed of well defined relatively small molecular weight molecules. Such a system may be particularly useful in future biochemical and biophysical studies to examine the structural changes occurring in both recipient DNA and donor DNA during genetic recombination.

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Membrane model for the circadian clock

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The biological clock may be a feedback system involving ions and ion-transport channels.

RHYTHMS which will entrain to a 24 h light/dark cycle and persist under constant conditions with a period of about 1 d are apparently ubiquitous among eukaryotic organisms¹. A striking feature of these circadian rhythms is that the 'free-running' period, exhibited when there is no entrainment by environmental cycles, remains close to 24 h over a wide range of temperatures. (The 'free-running' period is implied in all subsequent references to the period, unless specified otherwise. There is a survey of circadian rhythms in ref. 1.) Most investigators believe that these rhythms are generated by a cellular biochemical system and that, though the details may vary from species to species, some fundamental mechanism is common to all.

Nevertheless, the molecular elements of this biological clock have proved elusive. To facilitate the search for the oscillatory system, it is helpful to consider models. Two conceptually different approaches have emerged. One conceives of the clock as a 'tape' with discrete biochemical events occurring in a strict sequence. In molecular terms, this has been interpreted as sequential gene expression: transcription of DNA proceeds, one gene at a time, at a controlled rate, as described most explicitly in the chronon model of Ehret and Trucco². Another approach, pursued by Pavlidis³⁻⁵, Pye⁶ and Sel'kov⁷, conceives of the clock as a biochemical network with self-sustained oscillations arising from feedback within the biochemical system. This notion can be traced to the oscillator theories of Pittendrigh and Bruce⁸ and the experimental work of Chance and associates^{9,10}.

Our model incorporates the network idea, but identifies ions and membrane-bound ion transport elements with the biochemical clock, and thus with the primary oscillations. Feedback arises from the effect of ion concentrations on the activities of the ion transport structures, which in turn affect ion distributions. (Obviously changes in ion concentrations imply changes in trans-membrane ion gradients and vice versa. However, the regulatory effects of ions relate most immediately to concentrations while ion fluxes are most readily discussed in terms of gradients. Therefore we use both descriptions, although they are equivalent.) The rate-limiting kinetics—either of ion transport or of change in transport activity—are postulated to be a function of the lipids, which in natural membranes are known to be adapted to the environmental temperature^{11,12}. Temperature compensation of the free-running period is hypothesised to be a consequence of this. Light affects the clock by open-

ing a membrane ion gate which may itself be photosensitive or be triggered hormonally from a remote photoreceptor.

Three concepts on which this model is based thus deal with the role of ions and membranes, the effect of light, and the importance of membrane lipids. To provide a more complete picture, we propose that the circadian change in membrane transport activity may involve the migration of membrane-intercalated particles within the plane of the membrane, and that time-dependent cooperative phenomena may account for accurate timekeeping and homeostasis.

Role of ions and membranes

Chemical agents which might alter the phase or period have been sought in the hope of pinpointing the molecular components of the clock. A few have been known for some time (D₂O¹³, ethanol¹⁴, arsenite and *p*-chloromercuribenzoate¹⁵), but these gave no clear indication of a unique target. More recent experiments implicate ions and membranes. Valinomycin (a highly specific carrier of K⁺) causes phase shifts in two systems, the bean plant *Phaseolus*¹⁶ and the dinoflagellate *Gonyaulax*¹⁷. Ethanol may act by changing the ionic permeability of biological membranes¹⁸; ethanol effects have now been demonstrated in *Phaseolus*¹⁴, *Gonyaulax*¹⁷ and the isopod *Excirolana*¹⁹. Although ion pulses are ineffective in several systems, this may be attributable to regulatory mechanisms for maintaining ion balances. K⁺ pulses do phase shift the rhythm in isolated eyes of the sea hare *Aplysia*²⁰ and lithium lengthens the period in the plant *Kalanchoe*²¹.

Ion involvement is also indicated by the fact that phytochrome, which may act as a K⁺ gate^{22,23}, apparently mediates the effect of light on the free-running period in *Phaseolus*²⁴ (28.1 h in red, 24.7 h in far red, 26.3 h in darkness or light of other wavelengths). Electric fields, which have an effect on some rhythms²⁵, should only affect spatially ordered systems, such as membranes and their surrounding charge distributions.

Rhythms are phase shifted by light pulses and entrained by light cycles, and the free-running period usually varies with the constant light intensity²⁶. In our model, light acts by perturbing ion concentrations (probably K⁺, since K⁺ and valinomycin pulses phase shift like light). The photoreceptors coupling to rhythmic systems are hypothesised to be photosensitive ion gates, as in visual systems and plant phytochromes. This allows for close coupling between the photoreceptor and the clock in unicellular organisms; specifically, a K⁺ flux through the photoreceptor gate augments or depletes the K⁺ concentration directly. In higher systems,

hormones may mediate between a specialized photoreceptor and the other cells²⁷. Consequently, the coupling hormone should induce a permeability change (possibly specific for K^+) in its target membrane. In support of this is Rensing's finding that the moulting hormone ecdysone phase shifts the rhythm in isolated *Drosophila* salivary glands²⁷. In *Chironomus* salivary glands, ecdysone acts by increasing the K^+/Na^+ ratio in cell nuclei²⁸. Circadian rhythm photoreceptors can be very sensitive and fast-acting, characteristics typical of photosensitive ion gates. A 1 ms flash phase shifts the fungus *Pilobolus*²⁹; an extraretinal photoreceptor of the sparrow is sensitive to 0.1 lux of green light³⁰. Winfree has found 1,000 ergs cm^{-2} applied over 2 min to be sufficient to saturate phase shifting in the fruit fly *Drosophila*³¹.

If the clock is a feedback oscillator with ions on one hand and membranes on the other, it must involve circadian changes in transmembrane ion fluxes. These fluxes, both passive and active, should be mediated primarily by membrane proteins. Activity changes in these proteins may involve either synthesis and degradation or activation and inhibition. Since protein synthesis is probably not required to drive the clock oscillation (see later), the clock function presumably involves activation and inhibition of transport proteins. The kinetics of the clock then must reflect both the kinetics of this activation and inactivation and the kinetics of the transport processes *per se*. But these rates probably depend also on the characteristics of the membrane lipids.

According to the 'fluid mosaic' concept of membrane structure³², membrane proteins are intercalated into the lipid bilayer and can move in the plane of the membrane through the fluid lipid matrix. Because the kinetics of this protein migration will depend on the fluidity of the membrane lipids, this migration constitutes a lipid-dependent process for activation and inactivation of membrane protein functions. A mechanism for temperature compensation of circadian rhythms is immediately suggested. The lipid composition of membranes is controlled to maintain a definite fluidity independent of temperature^{11,12}. The kinetics of protein migration should depend on the viscosity of the medium, so lipid adaption should render this temperature-compensated. The limited data available³³ suggests that the activities of some membrane-bound enzymes are temperature-compensated by the lipid adaption phenomenon, so the kinetics of protein-mediated ion transport may also be compensated in this way. Lipid adaptation also compensates the rate of passive ion diffusion through the lipid bilayer³⁴.

Characteristics of rhythms

It has long been known that observed rhythmic processes may not be an integral part of the timekeeper. Specifically, their suppression does not affect the clock. The only unmistakably clock-related experiments are those that concern shifting the phase or changing the period of the rhythm. Only those data will be considered in the ensuing discussion of clock properties.

A 24 h period is not easily derived from the relatively rapid processes encountered in biochemistry. The chronon model² relies on a 24 h transcription and recycling sequence, but the biochemistry has not been sufficiently elucidated to evaluate the model's kinetic feasibility. A biochemical oscillator might have difficulty with 24 h periods; experimentally studied systems have periods of no more than 15 min⁸ so a 100-fold frequency reduction is necessary. To circumvent this problem, Pavlidis has suggested that coupled oscillators may oscillate as a group with periods much longer than the individual units⁵. Spatial inhomogeneity in the timekeeper reactions may also lengthen the period. However, such a large frequency reduction should be critically dependent on the coupling parameters, and it is difficult to

imagine a biochemical system capable of maintaining the coupling constants within the limits required for an accurate period. The membrane model, by drawing on slower processes, such as lateral diffusion of proteins within the membrane³⁵⁻³⁷, can achieve 24 h periods more easily.

To provide for temperature compensation of the period, the chronon model² postulates aqueous diffusion processes to be rate-limiting, but the negative temperature coefficients shown by the algae *Oedogonium*³⁸ and *Gonyaulax*³⁹ are not accounted for. The network model of Pavlidis and Kauzmann⁴ postulates reduced enzyme levels at higher temperatures, attributing negative temperature coefficients to overcompensation. However, it cannot readily explain dual mode temperature compensation, such as that seen in *Euglena*⁴⁰, which has a period-length Q_{10} of 1.0 when grown autotrophically, but a Q_{10} of 0.93 when grown mixotrophically. The membrane model can attribute this to the fact that fatty acids of *Euglena* lipids vary with culture conditions⁴¹; negative temperature coefficients may be a consequence of overcompensation in the lipid adaptation process.

Circadian rhythms can be phase shifted by temperature steps and entrained by temperature cycles. (By convention, a temperature step is a prompt change in the environmental temperature. A temperature pulse involves a step followed (usually 12 h later) by an opposite step returning the system to its initial temperature. The repeated application of temperature pulses (usually with a 24 h period) is called a temperature cycle.) In *Drosophila*, entrainment by 24 h temperature cycles follows directly from the form of the phase response curve for single temperature steps⁴². A temperature cycle apparently acts as a series of independent steps, implying that phase shifting due to a temperature step is completed within 12 h. Experimentally, the direction of the temperature change determines the direction of the phase shift. For a temperature step up, a phase advance occurs as if the clock had run fast for awhile; for a step down, a phase delay occurs^{40,42-47}. This indicates that the temperature compensation process requires several hours to complete. In our membrane model, the lipids must adjust to a change in temperature with a time constant on the order of hours. Lipid compensation requires about 4 h in stationary phase *E. coli*⁴⁸, and earthworms injected with an extract from cold-adapted earthworms change their lipid saturation within 3 h⁴⁹.

In some systems, the overt rhythm is lost at low temperatures and the mechanism seems to settle into a unique phase point; when the temperature is raised, the rhythm resumes with a phase defined by that time^{39,40,50}. The membrane model provides an interpretation: the temperature is below the adaptation range for the lipids in the membrane, which then will undergo a transition to a more condensed phase. This should cause the ion gradients to fall into the pattern characteristic of the unique phase point; it is interesting that in *Gonyaulax* both low temperature and bright light hold the system at the same point, CT 12. (Circadian time (CT) is defined by dividing the period into twenty-four equal portions. CT 0 is chosen as the beginning of the light period or its equivalent in a free-running system.)

To analyse the effects of light on rhythms, a simple two-parameter example is helpful. Assume that the rhythm involves just one ion and its associated transport pathways. The trans-membrane ion gradient, x , will oscillate as in Fig. 1d. Membrane transport, y , will also vary but, for simplicity, let it have only two states (Fig. 1b). Light has the effect of depleting the ion gradient since it opens an ion gate. Therefore, a light pulse dropping the gradient to x_0 may have two effects. Applied before CT 18, it will cause a phase delay because the membrane is in the active mode and x will repeat its accumulation. A light pulse given after CT 18 will cause a phase advance since the membrane is in the passive mode and x will skip ahead in its discharge.

This simple illustration will not give quantitative agreement with phase shifting data. For a proper analysis of the two-dimensional oscillator, the dynamical equations should be written as $dx/dt = f_1(x, y)$ and $dy/dt = f_2(x, y)$. Light of intensity, L , acts on the system to deplete the gradient x . Since this is a passive flux, it should proceed at a rate proportional to x and to some function of the light intensity $f_3(L)$. Therefore, dx/dt must be modified to include the light effect: $dx/dt = f_1(x, y) - x f_3(L)$. An analysis of a particular set of equations of this type quantitatively reproduces the effects of light on the *Drosophila* system including the influence of the light intensity on the period^{3,4}. While these equations are not unique in their ability to account for the data, they do establish the quantitative plausibility of this sort of system. Of course, any network model can be fit to these equations, but the phase shifting caused by the rapid destruction of x is particularly descriptive of the depletion of ion gradients.

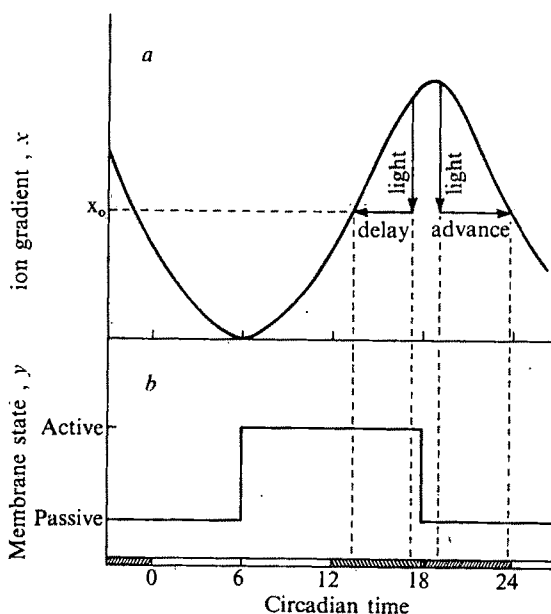


FIG. 1 Hypothetical oscillations in a simple one-ion system with only two membrane states. A light pulse applied just before CT 18 results in a phase delay since it brings the ion-membrane system to a state equivalent to that at about CT 13. Similarly, an identical light pulse just after CT 18 results in a phase advance. Maximum phase shifts are obtained at night.

The cellular clock couples biochemically to diverse expressed rhythms possessing various phase angles. Control of some processes, such as leaf movement in *Albizia*, can be readily attributed to ions²³, and rhythms in membrane processes such as photosynthesis, respiration, phototaxis and excitability could depend directly on ion variations. Some rhythmic processes, such as bioluminescence in *Gonyaulax*, involve, in part, variations in enzyme and substrate levels^{51,52}. Reconciling this with a membrane model is more difficult, although variations in enzyme levels could be achieved through ion-mediated activation and inactivation, without *de novo* synthesis. However, ions might also act by stimulating the production of inducers—perhaps for all rhythmically expressed proteins, perhaps only for specific rhythmically-expressed RNA polymerases. A diurnal variation in polymerases has been reported⁵³. The induction hypothesis allows genes to be regulated in groups, which would facilitate control of several complex processes associated with one circadian rhythm. Furthermore, this would make mRNA synthesis a driven rhythm rather than a part of the clock itself, explaining the failure of RNA synthesis inhibitors to phase shift the rhythm.

Speculations and implications

In considering the detailed structure of the membrane, we could imagine a simple model in which passive and active transport channels respond directly to changes in ion concentrations so that they flip back and forth into on and off modes. This probably has too fast a response time and would generate rapid oscillations as observed in mitochondria⁵⁴. Instead, we have suggested a requirement for lateral diffusion of protein in the membrane^{35,36}. Particles seen by freeze-fracture electron microscopy exhibit characteristic arrangements and sizes in differentiated membrane regions⁵⁵⁻⁵⁹ correlated with pH and other ion concentrations^{37,55,60,61}. We propose that these particle distributions can be regulated by oscillating ion concentrations and may themselves then regulate the ion concentrations to drive the oscillation. This is easy to imagine for structures such as cell junctions; ATPases and other enzymatic functions might also be activated and inactivated in this way. Furthermore, this process of constructing particle arrays should be relatively slow.

At the risk of conveying a simplistic and overly specific image, we present in Fig. 2 a diagrammatic version of our membrane timekeeper in the style of Singer and Nicolson's 'fluid mosaic' membrane³². This shows changes in both particle arrangements and sizes, caused by the ion distributions. The state of these particles, in turn, determines the direction and activity of membrane transport. While only one ion and a corresponding set of transport proteins is represented, other ions and other transport systems may be tied in, forming a system of coupled oscillators. A photoreceptor which serves as a K^+ gate is included in this picture; in other cases, this might be a hormone-triggered ion gate.

Circadian rhythms persist in enucleate cells^{62,63} and seem to be unaffected by many nucleic acid and protein synthesis inhibitors^{50,64-67}. Among such inhibitors only cycloheximide has been shown to affect a circadian rhythm⁶⁸ although the period change was small considering the degree of protein synthesis inhibition. The fact that inhibition of protein synthesis does not have a larger and more universal effect suggests that the clock keeps time relatively independently of precise enzyme levels. This is particularly compatible with the membrane model, since it does not require precisely balanced enzymic activities, especially to achieve temperature compensation.

A minimal dependence on stochastic fluctuations in enzyme levels has adaptive advantage in a clock mechanism and is an example of the principle of homeostasis: circadian clocks are insensitive to the perturbations they normally encounter⁶⁹. Time-dependent cooperative phenomena provide an attractive means for achieving timekeeping precision and homeostasis. The sigmoid curve characteristic of cooperative phenomena provides a more precise definition of time and is less sensitive to fluctuations in enzyme, metabolite or threshold levels than are functions based on non-cooperative processes. Indeed, regulation in biological systems is commonly associated with cooperativity^{70,71}. The formation of particle arrays ought to show time-dependent cooperativity, since it should be a nucleation process analogous to crystal growth.

A model should provide insight for further research. Our model makes specific and testable statements about the ionic nature of the fundamental oscillator, the action of light, the role of lipids in temperature effects, the insensitivity of the clock to fluctuations in enzyme levels, and the role of membrane-intercalated particles in maintaining self-sustained oscillations in ion concentrations.

In clock mutants, altered proteins might be involved in ion transport or lipid metabolism. Phenotypically, mutants might have altered ion transport properties, defects in membrane particle aggregation, or differences in lipid fluidity.

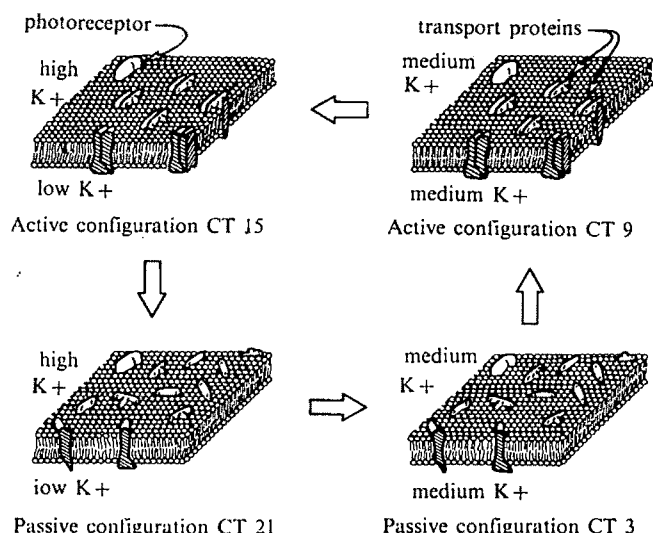


FIG. 2 Schematic representation of the way in which a fluid mosaic membrane³² might keep time. This illustrates the simple one-ion oscillator discussed earlier.

Membrane lipids can also be changed nutritionally or by inhibiting the lipid adaptation mechanism and changing the temperature. Changes in the period should correlate with changes in the lipids; the time course of lipid compensation in rhythmic cells should be determined.

This model predicts that the photoreceptor is membrane-bound and functions as an ion-gating device. In unicellular organisms, phase shifting with light should cause a change in the ion distributions. Mediating hormones in higher animals should produce changes in the ionic permeabilities of their target membranes. The response of membrane transport systems to ion concentrations *in vitro* can be analysed to see whether these systems are capable of self-sustained oscillations with 24 h periods.

Ferritin labelling and freeze-fracture electron microscopy should be useful in observing circadian rhythms in the arrangement of membrane-intercalated particles.

In summary, we have described a membrane model for the biological clock, which accounts qualitatively for many of its characteristic features. Ion concentrations and ion transport channels function as a feedback system to generate self-sustained circadian oscillations. Light acts on the rhythm either directly or through hormonal coupling to deplete trans-membrane ion gradients. Temperature compensation of the free-running period is a consequence of the temperature adaptation of membrane lipids. We have speculated that the timekeeping involves time-dependent cooperativity and the rearrangement of membrane-intercalated particles.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Infrared observations of Comet Kohoutek before perihelion passage

I REPORT in this letter broadband observations at 10 μm and 20 μm of the nucleus of Comet Kohoutek. These measurements were taken about 1 week before perihelion, while the comet was about 0.35 AU from the Sun. Table 1 summarises the results, which show that the comet was an intense infrared object, magnitude about -5 , and that the nuclear material responsible for the infrared emission heated up rapidly as the comet sped toward the Sun.

10- μm magnitude to be -4.56 relative to α Boo. (The extinction correction at 10 μm was 0.15 mag per air mass.) The uncertainty in my results arises primarily from the uncertainty in the standard magnitudes for α Boo and α Sco and also from the temporal variation in the infrared extinction. I estimate both these effects to contribute an error of no more than ± 0.03 mag in the final results.

Table 1 shows that as the comet grew brighter at about 10 and 20 μm , the colour difference decreased as expected from solar heating of the solid material in the nucleus. This colour change is reflected in the sharp rise of the colour temperature. The derived colour temperatures are above those expected from a grey-body sphere heated by sunlight at the same solar distance as the comet. (For example, at 0.33 AU the expected temperature is about 525 K.) Similar

TABLE 1 Infrared magnitudes of Comet Kohoutek

Date	[10 μm]	[20 μm]	[20 μm - 10 μm]	Colour temperature (K)	R(AU)*
December 19, 1973 1800 h UT	-4.51	-5.54	-1.03	500	0.39
December 20, 1973 1800 h UT	-4.74	-5.60	-0.86	700	0.36
December 21, 1973 1800 h UT	-5.03	-5.80	-0.77	750	0.33

* Taken from the Comet Kohoutek ephemeris of D. K. Yeomanis.

I obtained these measurements with the 1.3-m telescope at Kitt Peak National Observatory. The infrared detector was a germanium bolometer with a beam size of 24 arcs. The chopping secondary of the telescope was adjusted to give a beam separation of 38 arcs in declination. During the observations, I centred the beam on the position of peak infrared emission; this location defined the infrared nucleus of the comet. The magnitudes in Table 1 refer only to the infrared flux originating from the portion of the comet within the beam's area. As I found the comet's infrared emission extended over an area greater than 1 arc min, the comet's total integrated infrared magnitudes must be greater than those listed in Table 1.

All magnitudes are relative to the standard star α Boo. Since α Sco was conveniently close to the comet on the dates of observation, I also compared the comet to this star. The advantage of using α Sco was that it suffered from essentially the same atmospheric infrared extinction as the comet, so little correction in the relative magnitudes was necessary. I adopted -4.82 as the 20- μm magnitude of α Sco¹, and I measured its

excessive infrared colour temperatures have been noted for Comet Ikeya-Seki² and Comets Bennett and Tago-Sato-Kosaka³. This colour temperature anomaly is probably due to the spectral behaviour of the absorptive efficiency of the radiating grains in the nucleus². The data in this letter are not sufficient to identify the character of the grains but observations made as the comet streaks from the Sun may answer this important question.

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Detection of methyl cyanide in Comet Kohoutek

Most current models of comets assume that the nucleus is an icy solid body containing fairly complex parent chemical compounds which sublimate and dissociate to provide the radicals and simple molecules that have been observed in cometary spectra¹. Optical spectra show the presence of CN, CO⁺, CH, NH, OH and many others²; proposed parent molecules have included H₂O, HCN, HNCO, CH₃CN and H₂CO³. Previous attempts to detect H₂CO and H₂O were unsuccessful, probably due to excessive beam dilution^{4,5}. Here we report the first direct detection of a parent molecule, methyl cyanide (CH₃CN), at a wavelength of 2.7 mm in Comet Kohoutek (1973f). At the same time we were unable to detect either CN or CO at similar wavelengths.

Observations were made with the NRAO 11-m radio telescope at Kitt Peak, Arizona. The antenna had a half-power beamwidth of 67 × 63 arc s and a main beam efficiency of 0.55 for these observations. The first stage of the receiver is a double sideband Schottky-barrier crystal mixer with a 1,390 MHz IF; the overall system temperature is about 1,450 K single sideband. Spectral line data were taken using three filter banks: 256 contiguous 250 kHz filters, 50 contiguous 250 kHz filters, and 50 contiguous 100 kHz filters; all filter banks had the same centre frequency. The receiver was calibrated with a rotating chopper wheel; the calibration was checked by observing spectral lines of CO and CN with known strength in the galactic sources W51 and DR21. All data were reduced with the NRAO advanced on-line computing system⁶.

Table 1 is a summary of the observations. All values shown

sideband. After correction for the geocentric velocity expected, we calculated the rest frequencies given in the first two lines of Table 2. We are confident that the emission is from the comet nucleus only, as spectra from several days at different equatorial coordinates and different geocentric velocities all show the lines. Also, no lines were seen when we observed one arc min north, south, east and west of the nucleus. On December 3 we observed the same right ascension and declination as on December 1 to check for background sources; this also produced negative results. Finally, no emission was seen at several points along the comet's tail. Searches for CN and CO emission at several positions showed no detectable signals; the best upper limits are given in Table 2.

The two lines that we observed can be identified with 6,3 to 5,3, and 6,0 to 5,0 vibrationally excited transitions of CH₃CN at 110,709.55 and 110,712.22 MHz (ref. 7). Our calculated rest frequencies agree perfectly in line separation but are systematically 0.2 MHz high, or 0.54 km s⁻¹ low in geocentric velocity. This difference is probably not significant in view of the quoted errors. Methyl cyanide has previously been observed in Sgr A and Sgr B where its column density is comparable to that of CN (ref. 8).

The observed antenna temperatures may be used to calculate the optical depth and hence the column density of methyl cyanide. The exact lifetime of the CH₃CN molecule until photo-dissociation, and thus the distance from the cometary nucleus over which it should exist, are not known; a reasonable approximate value is 10⁴ km (refs 3, 9, 10). At a geocentric distance of 1.33 AU the region would subtend approximately 21 arc s and fill 0.06 of the antenna beam. The observed antenna temperature T_A is related to

TABLE 1 Observations of Comet Kohoutek (1973f)

UT Date (1973)	RA	1973.9 Dec.	Geocentric velocity (km s ⁻¹)	Geocentric distance (AU)	Heliocentric distance (AU)	Frequency range observed (MHz)
December 1	13 h 41 min	-20°10'	-34.1	1.36	0.87	(110,691-110,755) (113,472-113,536)
December 2	13 h 47 min	-20°37'	-33.2	1.34	0.85	(112,471-112,535) (115,251-115,315)
December 3	13 h 54 min	-21°06'	-32.1	1.32	0.83	(110,691-110,755) (113,472-113,536)
December 4	14 h 01 min	-21°34'	-31.0	1.30	0.81	(112,471-112,535) (115,251-115,315)
December 5	14 h 09 min	-22°02'	-29.8	1.29	0.79	(110,690-110,754) (113,471-113,535)

are averages over about 6 h of observation time daily. The ephemeris was supplied by T. Clark (Goddard Space Flight Centre) and calculated from elements published in IAU Circular No. 2588. The position of the comet was checked with an optical telescope attached to the antenna; it agreed with star positions from the FK4 catalogue within 10 arc s. The radio data were taken in a beam-switching mode—alternating integrations for 30 s on the source and on a position 30 arc min greater in right ascension. Two frequency ranges were covered simultaneously since the receiver responds to both sidebands. About half the data were taken looking through the fabric dome surrounding the telescope in order to protect the telescope surface from solar heating; the dome loss, about 40%, was taken into account when averaging the individual spectra.

Figure 1 shows spectra from the 100 kHz filter banks on December 1, December 5 and on both days combined; two lines are clearly visible in emission. They appear somewhat broadened by the filter response and by artificial smoothing. These lines were also detected in both 250 kHz filter banks. Also shown is the expected position of the CN line in the upper sideband. A shift in the local oscillator frequency showed that the observed lines were in the lower

the optical depth τ by

$$T_A = B\eta_n T_c \{1 - \exp(-\tau)\} \simeq B\eta_n T_c \tau \quad (\tau \ll 1) \quad (1)$$

where B is the beam dilution factor, η_n is the antenna main beam efficiency, and T_c is the temperature of the cometary gas. T_c is given by

$$T_c = \left(\frac{mc^2}{2k} \right) \left(\frac{\Delta\nu}{\nu} \right)^2 \quad (2)$$

where m is the mass of the molecule, c is the velocity of light, k is Boltzmann's constant and $\Delta\nu$ is the line half-width at 1/e intensity at the frequency ν . For $\Delta\nu = 0.12 \pm 0.04$ MHz and $\nu = 110,710$ MHz, $T_c = 263 \pm 160$ K for CH₃CN; the equilibrium temperature for a black body at a heliocentric distance of 0.83 AU is 306 K. Substituting into equation (1) $T_c = 263 \pm 160$ K, $T_A = 0.6 \pm 0.1$ K, $B = 0.06$, and $\eta_n = 0.55$ we obtain $\tau = 0.07 \pm 0.04$ for the 6,0 to 5,0 line. Similarly, the optical depth of the 6,3 to 5,3 transition is $\tau = 0.05 \pm 0.03$ and the CN and CO upper limits are $\tau \leq 0.06$ and $\tau \leq 0.15$, respectively, assuming the same T_c .

TABLE 2 Results

Observed rest frequency (MHz)	Molecule	Transition	Antenna temperature (K)	Intrinsic line half-width at 1/e intensity (km s ⁻¹)	Observed position
110,709.7 ± 0.2	CH ₃ CN	6,3 → 5,3	0.4 ± 0.1	0.32 ± 0.11	nucleus
110,712.4 ± 0.2	CH ₃ CN	6,0 → 5,0	0.6 ± 0.1	0.32 ± 0.11	nucleus
110,712.4	CH ₃ CN	6,0 → 5,0	≤ 0.25		tail
113,491.0	CN	1 → 0	≤ 0.20		(5' from nucleus)
115,271.2	CO	1 → 0	≤ 0.50		nucleus
					nucleus

The optical depth τ for a symmetric rotor such as CH₃CN is³

$$\tau = \frac{16\pi^2 N f_v h^{3/2} \mu^2 \nu^2 B C^{1/2}}{3c \Delta \nu (k T_c)^{5/2}} \frac{\{(J+1)^2 - K^2\}}{(J+1)} \cdot \exp(-W/T_c) \quad (3)$$

where h is Planck's constant, N is the column density (molecules cm⁻²), f_v is the fraction of molecules in the same vibrational state ($\sim 1/2$), B and C are the molecular rotation constants (both 9,199 MHz), μ is the dipole moment (3.92×10^{-18} e.s.u. cm), and W is the sum of vibrational and rotational energies of the upper level divided by Boltzmann's constant ($W = 500$ K). For the 6,0 to 5,0 transition, $\tau = 2.3 \pm 1.6 \times 10^{-18}$ N, and thus $N = 3.0 \pm 2.1 \times 10^{16}$ molecules cm⁻² averaged over the cloud diameter of 2×10^4 km. The 6,3 to 5,3 transition is theoretically weaker by a factor of 0.75, which agrees within errors with the observed ratio of 0.67. This transition gives $N = 2.9 \pm 2.1 \times 10^{16}$ molecules cm⁻².

Adopting the fluid dynamic model in which the molecular density is proportional to r^{-2} out to a critical radius r_c

where the molecules are destroyed⁴, then the number fraction b of CH₃CN molecules is given by

$$b = \frac{N v r_0}{4 Z R^2} \quad (4)$$

where v is the molecular velocity (4×10^4 cm s⁻¹), Z is the comet's gas production rate in molecules cm⁻² s⁻¹ rad⁻² and R is the radius of the icy nucleus. Substituting typical values of $Z = 3 \times 10^{18}$, $R = 2 \times 10^6$ cm and $r_0 = 10^9$ cm, we find for CH₃CN that $b = 0.025 \pm 0.018$.

In conclusion, the detection of methyl cyanide in the nucleus of Comet Kohoutek at a reasonable abundance has provided the first direct confirmation of the parent-daughter molecule hypothesis. Future observations of this comet as it passes perihelion are most desirable and should show changes in the density of such heavy molecules and their dissociation products.

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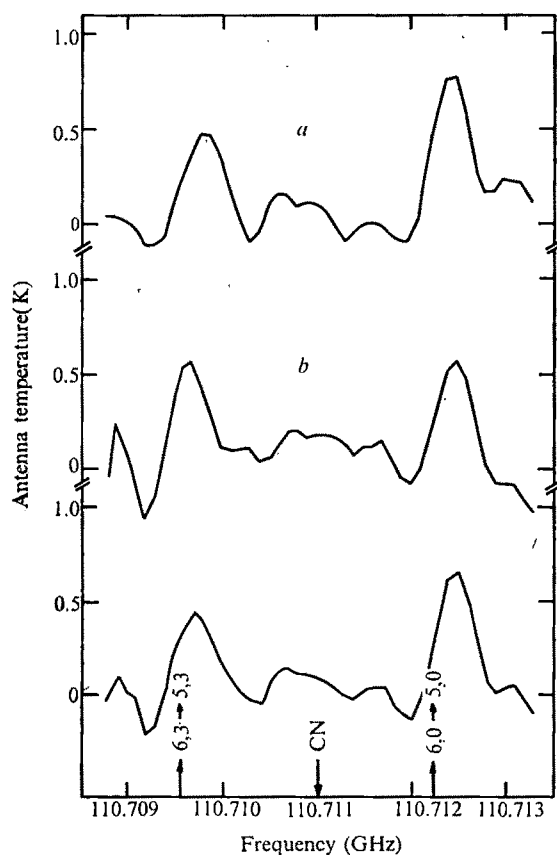


FIG. 1 Spectra of Comet Kohoutek (1973f) showing two emission lines attributed to CH₃CN: a, December 1, 1973; b, December 5, 1973; c, average.

Cosmic-ray energy spectrum from 10¹⁵ to 10¹⁸ eV

THE problem of establishing the primary cosmic-ray energy spectrum at energies $\geq 10^{15}$ eV, a region of great astrophysical interest, depends for its solution on the relation between the size and total primary energy of extensive air showers. This is because the experimental data are directly related to extensive air shower size spectra, which must then be converted to a primary energy spectrum. The necessary conversion depends, among other things, on the physics of the hadronic part of the cascade and on the primary composition in an energy region in which such information is only

available from extensive air showers themselves. The primary energy spectrum above 10^{15} eV is thus less well established than it is, for example, below 10^{12} eV, where direct measurements have been made, both of cosmic-ray primaries and of individual hadronic interactions.

In the absence of independent knowledge of primary composition and of properties of high energy interactions above 10^{12} eV, a demonstration that the relation of shower size to primary energy is, to some extent, independent of these features would be valuable. Such model independence is suggested by comparison of the calculation presented here, which assumes heavy primaries and Feynman scaling to relate shower size to primary energy, with calculations based on more conventional assumptions.

Various recent calculations (refs 1-3 and other papers presented at the Denver Conference) of extensive air shower (EAS) properties show that Feynman scaling for hadronic interactions appears to break down between $\sim 1,000$ GeV and $\sim 10^6$ GeV if the primary cosmic-ray beam is predominantly protons at EAS energies ($E \gtrsim 10^6$ GeV). It has been suggested, however, that if the primaries are predominantly heavy nuclei (for example, Fe) and if intranuclear cascading and an increasing p-air cross section are taken into account, then an approximate form of Feynman scaling (modified to allow for an increasing cross section) may hold from Intersecting Storage Rings (ISR) to EAS energies (refs 1, 2 and 2a). Here I report the use of a cascade calculation based on these assumptions to calibrate the Chacaltaya measurements of size against depth⁴⁻⁶ and obtain a primary energy spectrum from 10^{15} to 10^{18} eV. The spectrum obtained in this way is surprisingly close to that obtained from entirely different assumptions by the authors of refs 4, 5 and 6. (The authors of ref. 6 point out that, in view of the disagreement (around 10^{17} eV) between their results and those of refs 4 and 5, theirs are preliminary.)

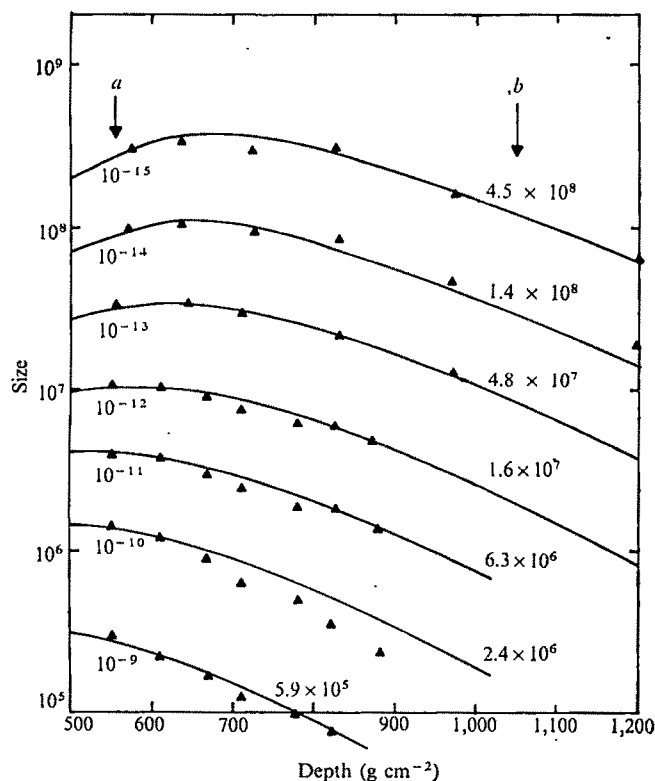


Fig. 1 Comparison between data and calculations of shower size against depth. Each calculated curve is normalised at one depth only (600 g cm^{-2}) to the data. *a*, Intensity ($\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$); *b*, corresponding total primary energy per nucleus, E_0 (GeV). ▲, refs 4 and 5.

Specifically, I assume pure iron primaries over the range of the Chacaltaya measurements, Feynman scaling⁷ from ISR data for the number distributions of pions produced in hadron-hadron interactions, and the model of Fishbane, Newmeyer and Trefil⁸ for intranuclear cascading. This picture of the primary composition is almost certainly oversimplified and possibly incorrect⁹ and the assumptions about hadronic interactions at EAS energies may have to be modified. Nevertheless, the quality of the fit to the Chacaltaya measurements of size against depth obtained in this framework makes it worth using it to convert the Chacaltaya size spectra to an energy spectrum.

In the spirit of this simple approach I treat an iron nucleus of total energy E_0 as a superposition of nucleons each of energy $E_0/56$. The size of the electromagnetic component of the shower is computed at various atmospheric depths as a function of E_0 using the cascade calculation described^{2a}.

The Chacaltaya shower development data⁴⁻⁶ were obtained by taking constant intensity cuts in the integral size spectra at various shower zenith angles, corresponding to different atmospheric depths. The minimum depth is thus the vertical depth at Chacaltaya, 550 g cm^{-2} . I obtain the energy calibration here by fitting the observed size at 600 g cm^{-2} to the calculated shower size at this depth for each intensity. The calculated shower development curves are compared with the data of refs 4 and 5 in Fig. 1. It is clear from the agreement between the curves and the data in Fig. 1 that a different choice of normalisation depth would produce

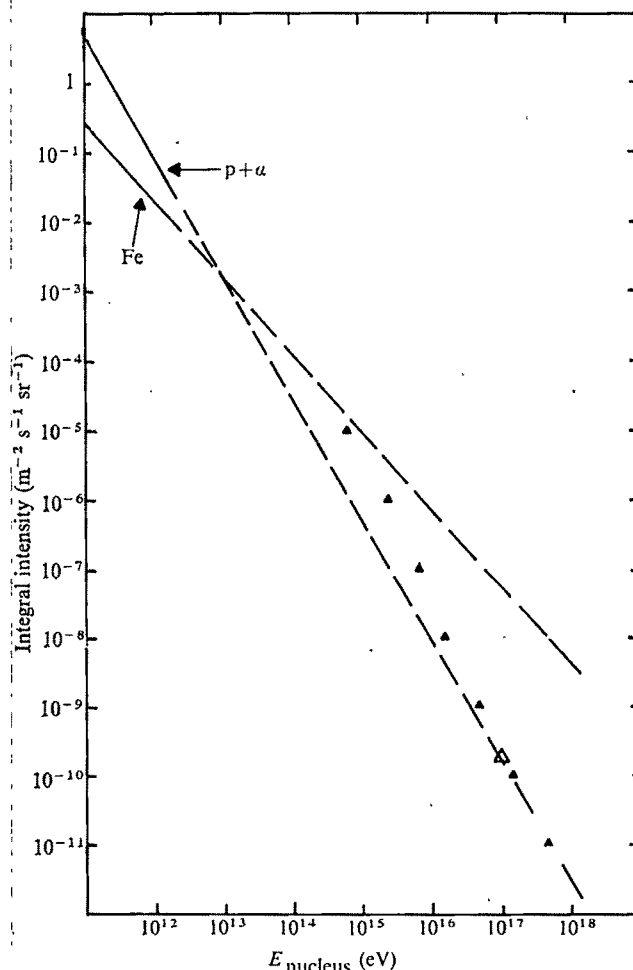


Fig. 2 Data points represent the primary integral energy spectrum obtained from Fig. 1 as described in the text. The normalisation obtained at 10^{17} eV from the EAS calibration of refs 4 and 5 is also shown as a ▲. ---, Extrapolations of Fe and $p + \alpha$ spectra measured at lower energies in balloon experiments (refs 10 and 11).

little change in the primary energy assigned to any of the intensities. Similar good agreement between calculated and observed shower development is also obtained for the data of ref. 6 (not shown here).

Figure 2 shows the integral energy spectrum of EAS primaries obtained from this analysis. A similar analysis has been carried out for the data of ref. 6. Both the magnitude and the slopes obtained agree within errors with those determined in refs 4-6 assuming predominantly proton primaries and using constant cross section and power law multiplicity for the number of particles produced in hadron-hadron collisions. In particular, the slope of the primary spectrum is steeper above 10^{15} eV than at lower energies ($\gamma \sim 2.2$ for the data of refs 4 and 5 and $\gamma \sim 1.8$ above 10^{17} eV for ref. 6). The similarity of primary energy spectra obtained under these two extreme sets of assumptions gives one confidence that the energy spectrum is not subject to appreciable uncertainties arising from ignorance of primary composition or of hadronic production mechanisms in this energy range (provided one uses a picture that fits the data on size against depth for EAS).

For comparison I show in Fig. 2 the data of the Goddard Space Flight Center on iron¹⁰ (plotted as number of Fe with total energy $>E$) and $p + \alpha$ primaries¹¹ (solid lines) and their extrapolations (dashed lines). Clearly, if the primary spectrum above $\sim 10^{15}$ eV is all iron, the slope of that spectrum is unlikely to have any relation to the mechanism¹² that may be responsible for the flat iron spectrum at low energy.

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Raman backscatter of Laser radiation from the stratosphere

RAMAN-shifted backscatter of laser radar radiation from atmospheric constituents in the troposphere has been reported¹⁻³. Signals from nitrogen have been detected up to heights of about 6 km and there have been indications of backscatter at even greater heights⁴. Oxygen and water vapour have also been the subject of tropospheric Raman investigations, generally using frequency doubled ruby (0.3472 μ m) as the laser transmitter.

Results outlined below show that the Mark II laser radar

system at the University of the West Indies, Kingston, Jamaica, can extend the range of Raman investigations on atmospheric nitrogen well into the stratosphere. This system has been described⁵ and is unchanged except for the introduction of new digital counting circuitry. A pulsed ruby laser transmits at a wavelength of 0.6943 μ m and the Raman return from nitrogen is received at 0.8284 μ m. The returning radiation passes through an interference filter (bandwidth 1.5 nm) on to photomultipliers and then, by way of a suitable pulse discriminating and amplifying system, to counters which give a digital display of the photon count for gated 2 km and 4 km intervals. Although there is some disadvantage in scattering cross section and photomultiplier sensitivity at these wavelengths, data obtained indicate that signals can be detected up to a height of about 45 km.

In order to examine statistically the performance of the system, the photon counts from the different gates have been related to atmospheric density values obtained from balloon measurements carried out by the local Meteorological Office and from the US Standard Atmosphere Supplements for 15° N⁶. The laser radar site is at Kingston (18.0° N) and is approximately 10 miles from the point at which the Meteorological Office sends up balloons. Comparisons have had to be made with the US Standard Atmosphere as the balloons used by the Meteorological Office burst in the region of 30 km. In addition, the accuracy of balloon measurements rapidly diminishes above 20 km.

The received photon count C is expected to be proportional to the ratio $(\rho_0 T_V T_D / h^2)$ where ρ_0 is the atmospheric density; T_V the atmospheric transmissivity at 0.6943 μ m, T_D the atmospheric transmissivity at 0.8284 μ m, and h the distance from transmitter to the effective point of scatter. As it is not possible to determine directly the constant of proportionality, the ratio $(Ch^2 / \rho_0 T_V T_D)$ has been normalised to unity at the gate interval 22-24 km. (This point was chosen for technical reasons associated with the equipment and photon counting method.) For perfect correspondence of C and ρ_0 , the ratios for all other gates should be unity within the limits of probable error. Figure 1a shows the photon counts obtained from an investigation on the night of August 25, 1973, when 2,200 laser firings were made, compared with atmospheric density values obtained from the US Standard Atmosphere Supplement (annual average).

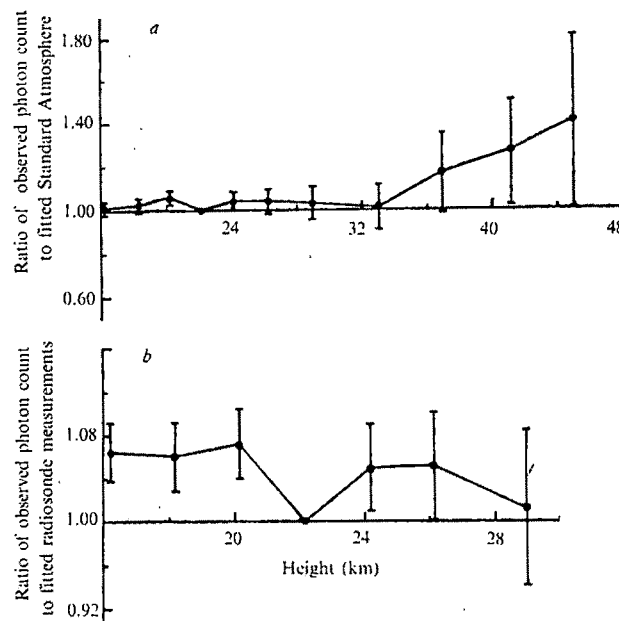


FIG. 1 Photon counts obtained on the night of August 25, 1973, compared to (a) US Standard Atmosphere Supplement for 15° N; (b) local radio-sonde measurements. The observations have been fitted at a height of 22 km.

This comparison is appropriate since the atmosphere at this latitude is remarkably constant. Figure 6 shows the same data related to radiosonde measurements made by the Jamaican Meteorological Office at 0700 h EST on August 26, 1973. In estimating the performance of the laser radar system, it must be noted that at a height of 28 km the pressure is in the region of 15 mbar, and radiosonde measurements of pressure can be in error by as much as 15%. There are also error contributions due to temperature and height measurements.

There is close agreement between the laser radar measurements and data from the Meteorological Office and the US Standard Atmosphere Supplement. The error bars show the standard deviation of the laser radar measurements based on the counting statistics. Most points are within one standard deviation of unity and none are much more than two distant from it. No allowance has been made for any possible errors in the radiosonde data. We think the results confirm that useful Raman signals from atmospheric nitrogen can be detected by the Mark II system up to a height of about 40 km. In the final gate (44–48 km) the signal-to-noise ratio was approximately unity whereas for the 36–40 km gate it was greater than 3.

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Caesium-137 as a water movement tracer in the St George's Channel

CALCULATIONS based on recent measurements of the distribution of ¹³⁷Cs in the St George's Channel show that the residual (non-tidal) flow is slightly lower than previous indirect estimates suggested. The contribution of turbulent mixing to the transport of dissolved and particulate material in these waters may also be calculated directly from caesium measurements.

Study of the isohaline patterns and of drifter movements shows that there is a residual flow of water from south to north in the Irish Sea system. It is, however, difficult to arrive at an accurate mean value for this flow because of the effects of turbulent mixing in modifying the isohaline pattern, and the effects of wind on surface drifters. Available current meter measurements and subsurface drifter experiments are insufficient in number to give long term mean values for such a large scale low velocity flow.

Knudsen¹ approached the problem by application of the continuity conditions for water and salt. Since he neglected the

TABLE 1 Field data used to calculate K_z and U

Survey		Southern boundary	Northern boundary	Caesium in channel (Ci)
May 1972	S	34.63	34.30	4,073
	C_s	1.8	8.7	
	$\frac{\partial C_s}{\partial x}$	0.04	0.1	
September 1972 RRS John Murray Cruise 11-72	S	34.75	34.25	3,802
	C_s	1.3	7.2	
	$\frac{\partial C_s}{\partial x}$	0.03	0.13	
February 1973 MV Researcher Cruise 02-73	S	34.77	34.36	3,634
	C_s	1.7	12.0	
	$\frac{\partial C_s}{\partial x}$	0.01	0.19	
June 1973 RRS John Murray Cruise 05-73	S	34.63	34.30	5,018
	C_s	1.7	16.0	
	$\frac{\partial C_s}{\partial x}$	0.01	0.21	

Salinities from Bowden⁴. Caesium values (May 1972) from Jefferies *et al.*⁶; others from unpublished data of this laboratory. Units: Salinity (S) parts per thousand by weight; ¹³⁷Cs pCi⁻¹ (equivalent to Ci km⁻²).

transport of salt by turbulent mixing the results he obtained (400 to 700 km yr⁻¹) are rather higher than those obtained by methods which take account of this process². Proudman³ correlated the passage of salinity anomalies at various observation points in the Irish Sea system and obtained a travel time corresponding to about 400 km yr⁻¹; Bowden⁴ utilised improved data to obtain a value of about 150 km yr⁻¹ by a similar method. Proudman⁵ derived an estimate for the ratio of the flow velocity to the diffusion coefficient normal to the flow direction by fitting a parabola to the observed isohalines between Holyhead and Dublin; this approach gave estimates of between 750 and 270 km yr⁻¹ depending on the value assumed² for the diffusion coefficient.

Bowden² extended Knudsen's method by including diffusive terms in the equation of continuity of salt. The continuity equations he obtained are as follows.

For water

$$X_1 U_1 + W = X_2 U_2 \quad (1)$$

(where X_1 and X_2 are the south and north boundary cross-sectional areas respectively (Fig. 1), U_1 and U_2 are the corresponding mean advective velocities (northwards positive) and W is the net fresh water input to the channel from precipitation, evaporation and runoff).

For salt

$$\rho_1 X_1 U_1 S_1 - \rho_1 X_1 \left(K_z \frac{\partial S}{\partial x} \right)_1 = \rho_2 X_2 U_2 S_2 - \rho_2 X_2 \left(K_z \frac{\partial S}{\partial x} \right)_2 \quad (2)$$

where ρ and S represent densities and salinities respectively and the second term on each side of the equation represents the mass flux of salt through the appropriate cross section by turbulent mixing. This equation may be simplified by assuming that $\rho_1 = \rho_2$ and K_z is constant: these are reasonable assumptions for the St George's Channel, where the north and south cross sections are similar.

Because K_z , U_1 and U_2 are unknown it is not possible to obtain a direct solution by considering only equations 1 and 2. To circumvent this problem Bowden² assumed K_z at the southern boundary to lie between 0 and 8.8×10^6 cm² s⁻¹. Using this assumption he estimated that U_1 lay between 126 and 610 km yr⁻¹ and U_2 between 111 and 438 km yr⁻¹.

Study of the movement of ¹³⁷Cs, released into the Irish Sea as a component of the low level radioactive waste pro-

duced at the Windscale plant of British Nuclear Fuels Ltd, provides a means to the direct solution of this problem. Caesium-137 is an almost ideal tracer for seawater movement as it has a long half-life (~ 30 yr), does not adsorb significantly on to sedimentary material and is easily detected at very low concentrations. Since the amounts of tracer added are several orders of magnitude less than the seawater concentration of natural (non-radioactive) caesium ($0.3 \mu\text{g l}^{-1}$), the presence of the tracer has no influence on the density or other physical properties of the seawater. It is, however, necessary to separate the tracer from the natural background activity of seawater due to ^{40}K (200 pCi l^{-1}) and to concentrate it for counting. In the present work this is done by adsorbing the tracer from up to 10 l of seawater onto a small volume (1 ml) of copper ferrocyanide. The retention of caesium is essentially complete (98%) whereas potassium is not adsorbed. Data for three complete surveys of the Irish Sea system have been obtained by this method (Table 1); the results for February 1973 are shown in Fig. 1. The results

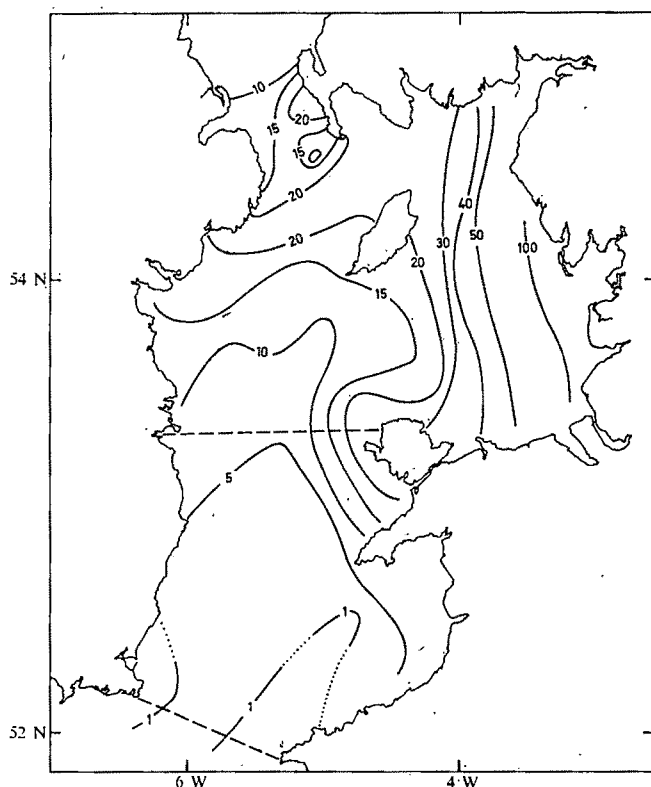


FIG. 1 Distribution of caesium-137 in the St George's Channel and Irish Sea during February 1973. Survey made on ICOT cruise, RS-02-73. Units are pCi l^{-1} . North and south boundaries of the St George's Channel are shown by dashed lines.

of an additional survey⁶ made during May 1972 have also been used in the present study. These analyses were carried out by a method broadly similar to that outlined⁷.

Since the concentrations of ^{137}Cs in the St George's Channel are not at steady state the continuity equation takes the following form

$$\begin{aligned} X_1 U_1 C_{s1} - X_1 K_x \left(\frac{\partial C_s}{\partial x} \right)_1 \\ = X_2 U_2 C_{s2} - X_2 K_x \left(\frac{\partial C_s}{\partial x} \right)_2 + P \end{aligned} \quad (3)$$

where P is the increase in ^{137}Cs within the channel volume during the time period under consideration. This quantity

may be obtained by integration of the ^{137}Cs values, shown in the survey contour charts, over the whole water volume. For this purpose the channel was divided into rectangles $10'$ latitude by $30'$ longitude (604 km^2); each was assigned an equivalent volume based on the mean depth and the fraction of the total area covered by the water. Measurements of the depth distribution of caesium⁸ show that in this region the concentration of caesium does not vary significantly with depth. Consequently in this study the surface value was assumed to apply to the whole water column.

Equations (1), (2) and (3) may be solved for the three unknowns

$$\begin{aligned} K_x = \{ W S_2 C_{s1} - W S_1 C_{s2} + P(S_2 - S_1) \} \left\{ \left(X_2 \left(\frac{\partial C_s}{\partial x} \right)_2 \right. \right. \\ \left. \left. - X_1 \left(\frac{\partial C_s}{\partial x} \right)_1 \right) (S_2 - S_1) - \left(X_2 \left(\frac{\partial S}{\partial x} \right)_2 \right. \right. \\ \left. \left. - X_1 \left(\frac{\partial S}{\partial x} \right)_1 \right) (C_{s2} - C_{s1}) \right\}^{-1} \end{aligned} \quad (4)$$

$$\begin{aligned} U_1 = \left\{ K_x \left(X_2 \left(\frac{\partial S}{\partial x} \right)_2 - X_1 \left(\frac{\partial S}{\partial x} \right)_1 \right) - W S_2 \right\} \\ \cdot \{ X_1 (S_2 - S_1) \}^{-1} \end{aligned} \quad (5)$$

$$U_2 = \{ X_1 U_1 + W \} X_2^{-1} \quad (6)$$

These equations may be evaluated numerically for the time period between each survey using the values given in Table 1 and the information on channel geometry and freshwater flow given in ref. 2. The results, which are given in Table 2, show excellent internal consistency and fall close to the range of values estimated by Bowden.

TABLE 2 Summary of results

Period	K_x $\text{km}^2 \text{ yr}^{-1}$	10^6 cm^2 s^{-1}	U_1 km yr^{-1}	U_2 km yr^{-1}
May to September 1972	8,130	2.59	129	113
September 1972 to February 1973	8,160	2.60	116	103
February to June 1973	8,980	2.86	141	124
Surveys before 1972				
Mean	11,600	3.7	.97	.88
Range	9,500-16,000	2.7-5.1	103-88	91-78

Bowden also considered other sections of the Irish Sea system to the north and south of the present area of study. Attempts to apply the continuity equation for caesium to the most northerly section (the Irish Sea proper) have shown that the assumption of constant K_x leads to anomalous results for this section. This is not surprising in view of the intense and complex mixing and advective processes in the North Channel (see Fig. 1) and the obvious dissimilarities between the north and south boundaries of this section. A further programme of study in this area is being undertaken. South of the St George's Channel (Celtic Sea) the radiocaesium levels are too low for reliable application of the present approach.

It is interesting that the total quantity of ^{137}Cs in the St George's Channel reflects the fact that about one third of the total ^{137}Cs input to the Irish Sea during 1972 was made in the August of that year (H. Howells, personal com-

munication). This 'pulse' apparently arrived at the northern boundary of the St George's Channel at about the time of the survey in February 1973 and shows as a sudden increase in total ^{137}Cs in the results for June 1973. The travel time of 8 to 10 months for passage through the Liverpool Bay system gives an apparent velocity of about 200 km yr^{-1} ; it is possible, however, that the mechanism of transport is predominantly diffusive rather than advective, since tidal mixing is very strong in this region.

In addition to the data used in the above calculations three other, less complete surveys are relevant. These were made as part of the research programme of the Ministry of Agriculture, Fisheries and Food Fisheries Radiobiological Laboratory, Lowestoft. The survey dates are September 1968, December 1969 (ref. 8) and June 1970 (D. F. Jefferies, personal communication). Although none of these surveys includes information on the southern boundary of the St George's Channel it is possible to make estimates of the values in this area from known North Atlantic fallout levels (1.0 to 0.3 pCi l^{-1}) and from the later surveys. This procedure permits the estimation of residual advective velocities and diffusion coefficients for four additional periods between surveys. The range of values of K_z so estimated is given in Table 2. The bias to slightly higher values shown by these estimates when compared with the calculated results may reflect the longer time periods involved, because advective processes operating for periods short relative to the time period concerned cannot be distinguished from diffusive processes and thus tend to increase the apparent diffusion coefficient.

The presence of ^{137}Cs in the Irish Sea system presents a unique opportunity to study large scale advective and diffusive processes over long time and distance scales. The present paper and that of Jefferies *et al.*⁶ illustrate only a few of the ways in which this fortuitous situation can be exploited to improve our knowledge. The near-shore transport processes here investigated control the movement and speed of all natural or artificial materials introduced into the waters around our coasts.

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An additional location of metalliferous sediments in the Red Sea

INTEREST in Red Sea brines and metalliferous sediments commenced in 1948 with reports of anomalously high bottom water temperatures in the vicinity of Atlantis II Deep¹. These reports were confirmed in the mid 1960s (refs 2-4) and subsequent investigations have revealed at least thirteen occurrences of brine pools and metalliferous sediment throughout the length of the Red Sea⁵⁻⁷. Detailed sampling of one deep, Nereus Deep ($23^\circ 10' \text{N}$, $37^\circ 15' \text{E}$), has indicated the presence of brines and metalliferous sediments of distinctive composition.

Nereus Deep is situated within the NW-SE trending median valley of the Red Sea, is 40 km long by 12 km wide, and is divided along its length by a saddle into two parallel basins (Fig. 1). Each basin contains a number of distinct depressions which either contain brine pools, or show evidence of having contained them in the past. The largest brine pool occurs in the deeper eastern basin filling two of the depressions, which appear to be separated by a low sill (Fig. 1). This brine has a chlorinity of 129.5‰ and a temperature of 30.2°C (ref. 7). In the western basin there is a smaller brine pool, which has a lower salinity, and may be present as a re-

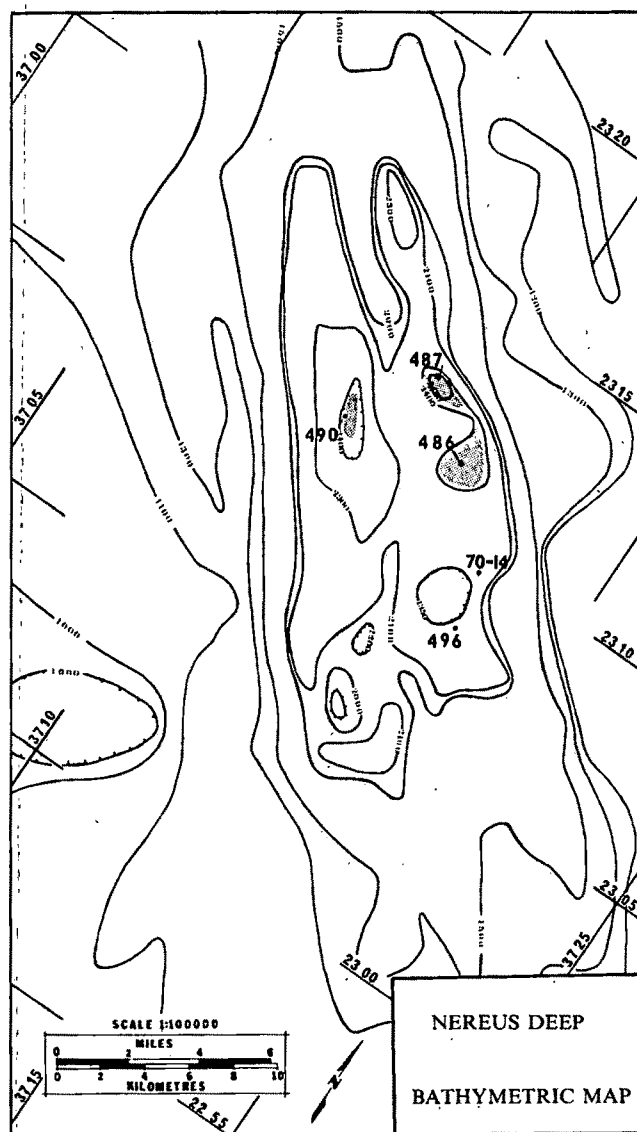


FIG. 1 Bathymetric map of Nereus Deep. Depths of water are in m.

TABLE 1 Average composition of metalliferous sediment layers from Nereus Deep

	70-14	70-14	70-14	70-14	496	496	486	486	486	486	487	487	487	487	490
	8-9	12-27	30-35	54-108	0-320	320-470	0-48	115-123	190-200	220-236	0-54	115-150	238-257	321-354	0-33
	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm
Fe%	29.70	26.80	20.10	29.40	20.74	40.35	6.70	2.70	17.00	3.00	4.80	10.90	12.55	5.20	5.83
Mn%	16.40	15.10	18.00	17.30	11.94	0.38	3.80	0.35	1.80	0.50	1.10	1.15	2.08	1.30	1.53
Zn p.p.m.	10,900	11,010	10,200	11,115	9,400	4,820	2,400	450	382	350	904	590	410	700	784
Cu p.p.m.	835	458	167	201	332	41	48	150	365	70	72	280	402	85	87
Ni p.p.m.	6	12	25	7	15		12	25	21	60	12	7	24	20	33
Co p.p.m.	15	12	15	12	14	8	16	30	30	30	16	10	32	25	39
Cr p.p.m.	20	15	15	15	42	33	14	20	18	12	19	12	25	20	44
Pb p.p.m.	370	380	200	495	314	540	42	50	20	40	92	53	64	70	60
* Hg parts per 10 ⁹	390	420	620	748	451	96	108	70	80	72	105	68	90	80	45
Ca %	4.20	5.60	6.00	3.80	2.88	0.88	10.20	18.00	7.00	18.00	11.50	12.00	10.70	15.00	17.00
† Salt %	15.0	14.5	14.0	14.5	14.5	18.5	48.0	30.0	47.0	23.0	47.0	48.0	41.0	30.0	14.3

* Core 70-14 was stored for 2 yr before analysis for Hg.

† Weight loss on washing.

sult of seepage or spillage from the main pool in the eastern basin.

Geochemical analysis of unwashed samples of sediment cores from the Deep has been undertaken for Fe, Mn, Zn, Cu, Pb, Ni, Co, Cr, Hg and Ca using atomic absorption techniques. This has confirmed the observed presence of several metalliferous layers within the sediments.

The most highly metalliferous sediments within the Deep occur in core 70-14 from the south-east slope (Fig. 1) and are apparently unrelated to any existing brine. This core contains four distinct layers of fine grained black-brown sediment of high water content. The true thickness of the deepest of these layers was not determined as the corer stopped within it, but 50 cm of metalliferous material was recovered and the possibility that they formed as a result of the oxidation of these black layers (Table 1) shows Fe to be very enriched, with Mn, Zn, Cu, Pb and Hg also enriched. These layers are chemically most similar to the 'manganite' facies of Chain Deep⁸. Core 496 (Fig. 1) taken from a greater depth in the south-east corner of the deep also contains 3.2 m of iron and manganese rich sediment (Table 1). Below this is at least 1.5 m of Fe-rich sediment.

The origin of the black manganese rich sediments is difficult to explain owing to the absence of any direct connection with existing brine. Possibly they were laid down in association with pre-existing brines, or result from diagenetic remobilization of previously buried manganese dioxide under reducing conditions. Perhaps more likely, however, is the possibilities that they formed as a result of the oxidation of dissolved manganese at the brine/seawater interface, with subsequent precipitation of manganese dioxide around and above the margins of the brine pool (H. Bäckér, personal communication).

Cores such as 486 and 487 from beneath the brine pools in the eastern basin (Fig. 1) show at least four distinct periods of brine influx (Table 1) during which fine banded metal-rich layers were deposited. Examination of the microfossil assemblages of these two cores has shown that the four metalliferous layers can be correlated (S. Ali, personal communication). In core 486 two of these layers are thinner and less metalliferous than the corresponding sediments in core 487, suggesting the sill between these two depressions may have inhibited brine flow from north to south. The brine precipitates have a high salt content and are enriched in Fe, Mn, Zn and Cu (Table 1).

The western basin seems to have had a much shorter history of brine deposition than the eastern basin, brine precipitates only being found in the upper 50 cm of the sediment column below the present brine pool. The salt and metal contents of these precipitates are much lower than in the eastern basin (Table 1), suggesting that the brines in the western basin may have been mixed with normal Red Sea water. The level of initial brine influx coincides with a zinc

and manganese rich layer, containing the maximum values of these elements in core 490 (Table 1).

Major points of interest in Nereus Deep are, therefore, first the occurrence of an appreciable thickness of iron and manganese-rich sediment containing various minor elements and, second, the possibility that, as in Atlantis II Deep^{8,9} sulphide layers rich in zinc, copper and lead may occur at greater depths. Large concentrations of mercury in the Deep (Table 1) support this view and may indicate hydrothermal enrichments of less volatile elements below.

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Crystal formation and growth in bivalve nacre

THERE are two theoretical explanations of the initiation and growth of the tabular aragonite crystals forming the nacreous layer in molluscan shells. The 'template' or 'matrix' hypothesis assumes that crystal nucleation and growth occur on the open surface of conchiolin membranes and may depend on active sites of this organic substratum². This implies a process comparable to epitaxy. In contrast, the 'compartment' hypothesis envisages that nucleation and growth occur within, and are controlled by, pre-existing hollow compartments of the organic deposits³. Two recent

scanning electron microscope (SEM) studies⁴ indicate that in gastropods there are no such compartments at the growth front of the nacreous layer. These studies are, however, not completely conclusive because of insufficient fixation of the samples. We have therefore studied the formation and growth of initial nacreous crystals in several species of bivalves.

We selected young individuals of *Pteria colymbus*, *Isognomon alatus*, *I. radiatus*, *I. bicolor* and *Brachidontes exustus* in which active shell growth was in progress. The molluscs were put in 6% solution of magnesium sulphate in seawater until the tissues were relaxed, then fixed by a 2% solution of glutaraldehyde in cacodylate buffer prepared in seawater for 4 h at room temperature.

After rinsing in buffer, the desired portions of the shells were cut off with the mantles attached. For transmission electron microscopy (TEM), they were dehydrated in an ethanol series, embedded in Spurr⁵ plastics, and sectioned with a diamond knife. Sections were stained with Reynold's lead citrate⁶ and/or uranyl acetate. The uranyl stain also served for decalcification.

For SEM, shells were dehydrated using the critical point method and the mantles pulled off to expose the inner shell surfaces; the samples were then coated with gold-palladium under vacuum.

Observations with the SEM confirmed that, in contrast to gastropods^{4,7} the nacreous layers of these bivalves are formed in a step-like pattern (Fig. 1a), each step corresponding to the growth edge of a nacreous lamina. In front of each lamina appear widely scattered crystals which are in the initial stage of formation. They grow by fast lateral and slower vertical accretion, acquiring first discoidal or sub-hexagonal habits and later fusing into single groups which finally merge to form a coherent nacreous lamina.

Transmission electron microscopy observations of the sections of mantle/shell preparations from semi-mature laminae (laminae crystals of which have not completely fused) reveal a situation similar to that described by Bevelander and Nakahara in other bivalve species⁸. Gaps are often found

between some of the crystals or crystal groups of the latest nacreous lamina. Some of the gaps contain traces of poorly condensed shred-like organic material (Fig. 1b, c). In addition, one or more sheet-like formations of condensed organic material have been observed between the microvilli of the mantle epithelium and the inner face of the nacreous layer. The innermost sheet may correspond to the mucous lining of the microvilli and the other formations may be independent membranous sheets. Examination of larger parts of the inner shell surface with the SEM has shown that such sheets extend over several nacreous steps (Fig. 1a). Thus, in the initial parts of the nacreous laminae these sheets are not strictly parallel to the surfaces of the laminae.

Near the edge of the shell is an organic lining which occupies the junction between the prismatic and the nacreous layer. It extends adorally beyond the growth front of the nacreous layer and covers part of the inner face of the prismatic layer.

The earliest initial stages of nacreous crystals appear on the surface of this lining. In some cases this occurs preferentially in the furrows representing the borders of the underlying prisms (Fig. 1d). A short distance behind they become fused to form the first coherent aragonitic lamina. In some cases the membranous sheets reach far enough forward to cover the growth front of the nacreous layer or even the inner face of the prismatic layer (Fig. 1d).

Our observations have shown that 'compartments' in the functional sense attributed to this term do not exist. In the semi-mature nacreous lamina the gaps covered by the membranous sheet are neither spaces controlling crystal formation and growth nor the result of crystal dissolution⁴ but simply places where the lateral fusion of the nacreous crystals has not been completed. They are certainly not compartments in the sense of the compartment hypothesis. On the other hand, the spaces between two adjacent horizontal membranous sheets as observed by Bevelander and Nakahara could be called compartments if the meaning of this term is restricted to a purely descriptive sense. But it seems questionable whether they control crystal growth.

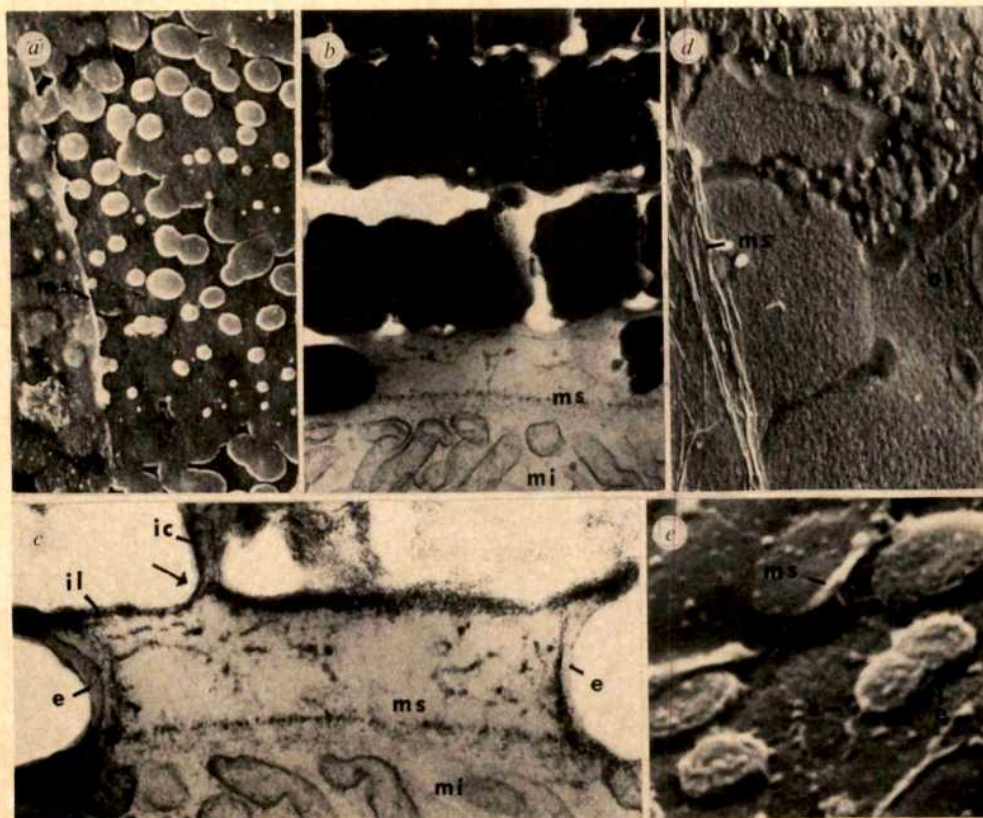


FIG. 1 Transmission and scanning electron micrographs of growing nacre in bivalves. a, Growing edges of three consecutive nacreous laminae in *Pteria colymbus*, all covered by a membranous sheet removed at the right side. Vertical view. $\times 1,680$. b, Gap in an incompletely fused lower-most nacreous lamina of *P. colymbus*. Vertical section. $\times 35,952$. c, Same. Note the organic envelopes of the crystals and the junction in the intercrystalline conchiolin membrane (arrow). Vertical section, decalcified. $\times 52,248$. d, Growth front of the nacreous layer (upper parts) covering the prismatic layer (lower parts) in *Isognomon alatus*. Note the organic lining underlying the nacre and the membranous sheet covering both layers and removed at right sides. Vertical view. $\times 336$. e, Surface of a growing nacreous lamina in *P. colymbus*. Note the organic envelopes of the crystals and the covering membranous sheet removed in the lower parts. Oblique view. $\times 6,720$. e, organic envelope of nacreous crystals; ic, intercrystalline conchiolin membrane; il, interlamellar conchiolin membrane; mi, microvilli; ms, membranous sheet; ol, organic lining underlying the nacreous layer.

It is true that they contain shreds of organic material in the initial state of consolidation and may, therefore, represent a separate micro-environment enclosing a modified pallial fluid³. Crystal formation and growth, however, seem to be rather independent as each of the initial nacreous crystals has its own organic envelope (e in Fig. 1c) which is independent of the organic sheets of the compartment (Fig. 1e). It is also inconceivable that the invariant vertical distance of the membranous sheet from the surface of the preceding nacreous lamina should be responsible for the uniform thickness of the newly formed mature crystals or lamina. Since this sheet is not consistently parallel to such a surface, its distance is not constant. We, therefore, conclude that the uniform thickness of the nacreous laminae cannot be attributed to the membranous sheet.

According to our observations initial and growing nacreous crystals are covered by a rather "tightly fitting organic envelope"⁴ consisting of one or several very fine linings (Fig. 1c, e) which are somewhat more conspicuous near the base of the crystal (Fig. 1e). During the lateral fusion of the crystals to form a mature nacreous lamina the adjacent lateral portions of the envelopes obviously merge (Fig. 1c) to become Gregoire's "intercrystalline conchiolin membrane". Such fusion of parallel organic membranes of identical structure and composition leaves no traces of a separating boundary. In our case, however, it is implied by the occasional preservation of their junction (arrow in Fig. 1c). The lateral fusion of the upper portions of the crystal envelopes results in the formation of a horizontal organic lamellar complex in which the original separations may remain detectable (arrow in Fig. 1c). This complex and the inner membranous sheet of the compartment forms Gregoire's "interlamellar conchiolin membrane" (see ref. 8).

The tightly fitting envelope of the growing crystals could easily be mistaken for a single membranous sheet belonging to the compartment and draped over the crystals following a collapse of the compartment during the preparation. Such an interpretation is, however, unlikely because the samples have undergone proper fixation and critical point dehydration. Finally it is invalidated by the observation that the envelope extends far under the latero-basal parts of the crystal (Fig. 1c).

There is, of course, the question of how this envelope is able to increase its surface as the crystal within it grows. It is, however, not a rigid structure. Since conchiolin membranes of the nacreous layer represent a network of tiny fibrils (see ref. 8) they can easily be stretched while new fibrils are added to fill the opening meshes. A good model is the avian eggshell membrane which is stretched as the egg grows in the isthmus.

Another question is why the organic envelope does not actually inhibit further growth of the crystal. There is still no answer to this but further investigation may show perforations or permeability of this membrane. Finally, why does the vertical growth of adjacent crystals cease at exactly the same level so that the mineral lamina resulting from their lateral fusion is uniform in thickness?

The evidence presented here shows that the formation and growth of nacreous crystals occur independently of the existence of the compartments from which these crystals are separated by individual tightly fitting organic envelopes. Since this envelope does not delimit a pre-existing space it is not a compartment in the sense of the compartment hypothesis. According to our observations the crystals at the growth front of the nacreous layer are formed on the surface of the organic lining which advances over the inner face of the prismatic layer. At the growth edges of subsequent nacreous laminae such initial crystals are formed on the surface of the interlamellar conchiolin membrane of the preceding nacreous lamina. Thus in both cases crystal nucleation occurs on the surface of an organic substratum,

an observation compatible with the template hypothesis.

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Possible cosmic dust origin of terrestrial plutonium-244

THE discovery of ²⁴⁴Pu by Hoffman *et al.*¹ in bastnaesite, a Precambrian rare earth fluorocarbonate ore from the Mountain Pass deposit in California, has raised the question of whether the so-called extinct nuclide, with a half life of 82 m.y., is part of the extant (or almost extinct) nuclide group or whether it was brought to Earth from a cosmic-ray source before the formation of the ore^{1,2}. I have reinvestigated the implications of its present existence in nature in terms of three possibilities: (1) survival of the primeval ²⁴⁴Pu, (2) influx as a heavy cosmic-ray component, and (3) inflow as a cosmic dust component from supernova remnants. The result shows that there are some difficulties with the first two possibilities, both of which were thought to be plausible at the time of the discovery. Although it is still a speculative proposition at present that any part of the cosmic dust falling on the Earth originated outside of the Solar System, a calculation suggests that the ²⁴⁴Pu may have been carried by such dust. The ¹²⁹Xe excess detected by Srinivasan *et al.*³ in an old Australian iodyrite, AgI, is also not inconsistent with the present hypothesis.

The observed amount of ²⁴⁴Pu, 2.0×10^7 atoms, in the 8.5 kg of ore reported in ref. 1 would correspond to an average terrestrial abundance of 2.0×10^{-24} g g⁻¹ or an average crustal abundance of 1.0×10^{-22} g g⁻¹, if the enrichment factor of plutonium was the same as that of cerium during the formation of this ore. The cerium enrichment factor is stated in ref. 1 to be a factor of the order of 5×10^5 greater than its average terrestrial abundance, or about 10^4 greater than its abundance in the Earth's crust. The former value for plutonium concentrations is consistent with an upper limit of 3×10^{-22} g g⁻¹ for the average terrestrial value obtained

earlier by Fields *et al.* from a 1.6 kg gadolinite from the Iveland district of southern Norway⁴.

An amount of 7.8×10^{-27} g ^{244}Pu g⁻¹ is expected for the average terrestrial abundance from the primaevial meteoritic abundance of $[^{244}\text{Pu}/^{238}\text{U}] = 0.013$ (ref. 5) and $[\text{U}] = 2.0 \times 10^{-2}$ p.p.m. (ref. 6 and references cited therein). This expected amount is such that the chemical enrichment of plutonium would have to be about 260 times more than that of cerium, as discussed in a similar way by the original investigators¹. The requirement of this high enrichment of plutonium (1.3×10^3) relative to the cerium enrichment (5×10^5) seems to be difficult to accept in terms of the possibility of a primaevial origin in view of the known chemical similarities between the two elements. Alternatively, the observed concentration may reflect a higher initial abundance of ^{244}Pu at the time of the Earth's formation (4.56×10^9 yr ago) relative to the initial chondritic abundance. A solution to the argument must await a substantiation of the discrepancy by further studies on other samples of bastnaesite.

Price *et al.*⁷ summarised the heavy cosmic-ray track data obtained by balloon flights up to 1971 to be two tracks due to $Z = 96 \pm 2$ per $2,110 \text{ m}^2 \text{ h}$. It may be that an order of one track per $2,110 \text{ m}^2 \text{ h}$ is recorded by ^{244}Pu , which leads to a rate of influx of 5×10^{-33} g ^{244}Pu cm⁻² s⁻¹. Assuming a steady state cosmic-ray source and a factor of survival against nuclear disintegration during passage through the atmosphere of 0.1 (which may be too high), the amount accumulated during the ^{244}Pu mean life (1.2×10^8 yr) results in a concentration of 6.5×10^{-25} g g⁻¹ in the 10 km of the Earth's crust. The assumptions involved are the same as those in ref. 1. But the age of $\geq 5.5 \times 10^8$ yr for the bastnaesite used by Hoffman *et al.* needs to be revised to a more reasonable one of 1.4×10^9 yr in the light of a recent study by Fleischer and Naeser² and the biotite age determined by Lanpher⁸. The expected concentration of ^{244}Pu from cosmic rays is thus reduced to 5×10^{-30} g g⁻¹ in the crust which requires an enrichment factor for plutonium of about 2×10^{11} (20 million times that of cerium in the Earth's crust). A lower influx of cosmic-ray ^{244}Pu and a smaller survival factor for passage through the atmosphere would require a higher enrichment factor for plutonium in this bastnaesite.

The present estimate of cosmic dust influx is around 10^5 tons per year over the entire Earth's surface (S. Tanaka and colleagues, unpublished). The life history of the dust in space is not well known in spite of great concern to many investigators. But a rough estimate using the Poynting-Robertson effect, friction with interplanetary gases, light pressure and gravity as the forces exerted on the dust shows that a dust particle of less than $1 \mu\text{m}$ at 10^4 AU falls down to the Sun within a few thousand million years⁹. Assuming that some of the dust, say 10% of the influx on to the Earth, originated in frequent supernova explosions in our Galaxy and that the dust material is chondritic, with $[^{244}\text{Pu}/^{238}\text{U}] = 0.013$ and $[\text{U}] = 2.0 \times 10^{-2}$ p.p.m., the steady state influx rate of ^{244}Pu is expected to be 1.7×10^{-26} g ^{244}Pu cm⁻² s⁻¹. Using essentially the same assumptions as in the above cosmic-ray calculation, a concentration of 1.6×10^{-22} g ^{244}Pu g⁻¹ in the Earth's crust is deduced. This is almost the same as the average concentration in the crust obtained from the observation of Hoffman *et al.* using an enrichment factor for plutonium equal to that of cerium. The small difference of a factor of 0.6 may be not important, considering the present state of knowledge of ^{244}Pu behaviour from its place of birth to the ore formation, that is, the ^{244}Pu formation in supernovae, its distribution into interstellar medium, the condensation into dust grains, the dust collection efficiency by the Earth, the ^{244}Pu geochemistry and concentration mechanisms and the possible small differences in these properties between plutonium and cerium.

The reported ^{129}Xe excess of $(2.2 \pm 0.2) \times 10^{-12}$

cm³STP/g of natural iodyrite can be ascribed to the *in situ* decay of naturally occurring ^{129}I and leads to iodine with the isotopic composition of $3.3 \times 10^{-15} \geq ^{129}\text{I}/^{127}\text{I} \geq 2.2 \times 10^{-15}$ if extrapolated back to some 50 m.y. ago when the ore was formed³. The concentration of ^{129}I in the Earth's crust is thus expected to be $(1.2 \sim 13) \times 10^{-22}$ g g⁻¹ from the enrichment factor of $(5 \sim 36) \times 10^5$ based on the iodine content of 25% in the ore and the crustal iodine abundance of 0.07 to 0.5 p.p.m. (refs 10, 11).

If the cosmic dust carries 0.1 p.p.m. of iodine with an isotopic composition of $^{129}\text{I}/^{127}\text{I} = 1.1 \times 10^{-4}$ (chondritic values¹²), the global influx rate of 1×10^4 ton yr⁻¹ would correspond to an influx of 7×10^{-28} g ^{129}I cm⁻² s⁻¹ and the same processes mentioned above for ^{244}Pu in the dust would yield a concentration of 2×10^{-19} g ^{129}I g⁻¹ in the crust. This value is two to three orders of magnitude higher than the expected one from the ^{129}Xe observed in the iodyrite.

Iodine is known to concentrate in hydrosphere and biosphere rather than in lithosphere. Even in the crustal materials the iodine contents varies widely from type to type of rocks, for example 0.007 ~ 0.5 (typically 0.046 ~ 0.1) p.p.m. in igneous rocks which are the major crustal rock, 7 ~ 38 (typically 10) p.p.m. in shales which are the major sedimentary rock and 10.9 ~ 49 (typically 50) p.p.m. in deep sea sediments^{11,12}. The variation of iodine concentration between the Earth's surface and deep seated rocks thus ranges over factors of $10^2 \sim 10^4$ which compare reasonably well with the difference of ^{129}I between the expected values from cosmic dust and from the observed ^{129}Xe excess in the iodyrite.

Further contribution of ^{129}I to the Earth's constituents is expected from spontaneous and neutron-induced fission of uranium in rocks^{13,14}, spallation reactions on xenon in the upper atmosphere¹⁵, and neutron and muon reactions on tellurium^{16,17}. An estimate of the additions from these reactions has been obtained by Edwards and his coworkers to be about 2.2×10^{-12} for the marine $^{129}\text{I}/^{127}\text{I}$ ratio, which is within a factor of less than about ten of their experimental values for the Long Beach California brines, ranging from 5×10^{-12} to 2×10^{-11} , for assumed equilibrium ratios with hydrospheric and atmospheric processes at the time of trapping of ^{129}I (ref. 18). These results suggest an incomplete mixing of ^{129}I of the different origins with ^{127}I in various parts of the Earth.

An experimental search for ^{244}Pu in deep sea sediment would be feasible if the present hypothesis is valid. Further studies along this line are under way at our laboratory.

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New look at the lead isotope growth curve

THE isotopic composition of lead changes with time due to radiogenic production from isotopes of uranium and thorium. Two lead isotopes are produced from uranium, ^{206}Pb from ^{238}U , and ^{207}Pb from ^{235}U . The paired U-Pb decay schemes are particularly useful since geochronological information can be derived even when there has been a chemical fractionation of U from Pb at some time during the history of the sample. For example, the $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ ratios can be measured in a galena (PbS) sample; by assuming that the parent material for the galena had suffered no chemical fractionation of U from Pb from the time of formation of the Earth until production of the galena, one can calculate a 'model' age of formation for the galena. This model is referred to as single stage growth of lead and can be characterised by a μ value [$(^{238}\text{U}/^{204}\text{Pb})$, as measured today] for the parent U-Pb system.

Stanton and Russell¹ suggested that conformable lead ore bodies may be expected to contain lead which would meet the conditions for single stage growth. They found that ores of different ages fitted approximately to a single growth curve and proposed that this curve represented the development of the lead isotopic composition of the lower crust or upper mantle with time. Using high precision analytical techniques Stacey *et al.*² redetermined the single stage growth curve and found that galena samples ranging in age from 0 to 3,100 m.y. fit a curve with $\mu = 9.09 \pm 0.06$.

The calculation of a single stage growth curve depends on a number of variables. First, the parent material for the galena must conform to the requirements of a single, chemically closed U-Pb system. Second, we must know the time at which the U/Pb ratio of the Earth became fixed (commonly referred to as the age of the Earth). Third, we must know the isotopic composition of lead at the time of formation of the Earth and, fourth, we must have accurate values for the decay constants of both uranium isotopes. We will assume for the moment that the first condition is met and look at the effect of the other variables on the calculation of model ages and model μ values.

The geological complexity of the Earth makes a direct measurement of its age of formation impossible. Using lead isotopes Tatsumoto *et al.*³ have measured the ages of several meteorites. The oldest age found was 4.57×10^9 yr (using the new uranium decay constants discussed below). This age is the same as the model lead age for the moon³, and provides the best estimate available for the age of formation of the Earth. Any change in the age of the Earth has the opposite effect to a change in the uranium decay constants since the two terms in the lead isotope growth equations are multiplied together [for example $(^{206}\text{Pb}/^{204}\text{Pb})_t = (^{206}\text{Pb}/^{204}\text{Pb})_{T_0} + \mu (\exp(\lambda T_0) - \exp(\lambda t))$].

The decay constants of uranium were recently determined using modern analytical techniques⁴. The new decay constant for ^{238}U is 0.92% larger than the previously accepted value⁵, whereas that for ^{235}U is 1.28% higher. For directly measured $^{206}\text{Pb}/^{207}\text{Pb}$ ages, such as the meteorite ages discussed above, the effect of the change in decay constants is rather small. At 4,500 m.y. the age difference resulting from recalculation of data with the new constants is -1.4%; at 3,000 m.y., -1.5%; at 1,500 m.y., -1.7% and at 500 m.y., -2.6%. Only at very young ages does the age difference become large. The situation for model ages, however, is quite different. Recalculation of a galena model age using the new decay constants results in a lowering of the model age by 200 m.y. at 3,000 m.y. and 350 m.y. at 600 m.y. model age. Although the largest shift in model age occurs for young samples, the biggest change in model μ values is for old samples. At 3,000 m.y. the newly calculated μ value will be 15% lower than the old value but at 600 m.y. the change is 8%.

Tatsumoto *et al.*³ reported new measurements of Canyon Diablo troilite lead isotopic compositions which are the most primitive yet found. Use of their troilite composition in the model age equation (rather than the values reported by Oversby⁶ results in a decrease in model age of about 100 m.y., regardless of the age of the sample, and lowers the model μ value by 2% for young samples, ranging up to a decrease of 6% for the oldest samples.

The combined effect of replacing all of the previously used physical constants in the model age equations by the more precise new parameters is a large shift in model ages and model μ values for all samples. The oldest samples have their model ages lowered by 300 m.y. (about 9%) and their model μ values decreased by about 20%; for the young samples, the model ages are lowered by 450 m.y. (50%-100%) and the model μ values are decreased by 10%.

Table 1 shows a selection of data from Stacey *et al.*²; the model ages and model μ values were calculated using the new parameters discussed above. Only the two oldest samples have model ages which are in reasonable agreement with the geological age of the surrounding rocks. These samples, which are our best candidates for single stage leads, have quite low model μ values. The youngest samples, whose model ages are about 500 m.y. younger than the age of the surrounding rocks, have considerably higher model μ values. This implies

TABLE 1 Model ages and model μ values for galenas

Locality	Geological age of surrounding rocks (m.y.)	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	Model age (m.y.)	Model μ value
Bathurst	460	18.20	15.65	Negative	n.a.
Balmat, New York	1,100	16.93	15.50	586	8.13
Broken Hill	1,500-1,700	16.01	15.40	1,210	8.12
Flin Flon	1,865	15.34	15.14	1,480	7.79
Geneva Lake, Ontario	2,500-2,700	14.00	14.87	2,340	7.89
Manitouwadge	2,700	13.21	14.40	2,630	7.39
Barberton	3,100	12.46	14.08	3,080	7.56

Isotopic composition data and ages of surrounding rocks from Stacey *et al.*². Constants used in model age calculation: $T_0 = 4570$ m.y.; uranium decay constants from Jaffey *et al.*⁴, $^{238}\text{U} = 0.155125 \times 10^{-9} \text{ yr}^{-1}$, $^{235}\text{U} = 0.98485 \times 10^{-9} \text{ yr}^{-1}$; initial lead isotopic composition³, $^{206}\text{Pb}/^{204}\text{Pb} = 9.307$, $^{207}\text{Pb}/^{204}\text{Pb} = 10.294$.

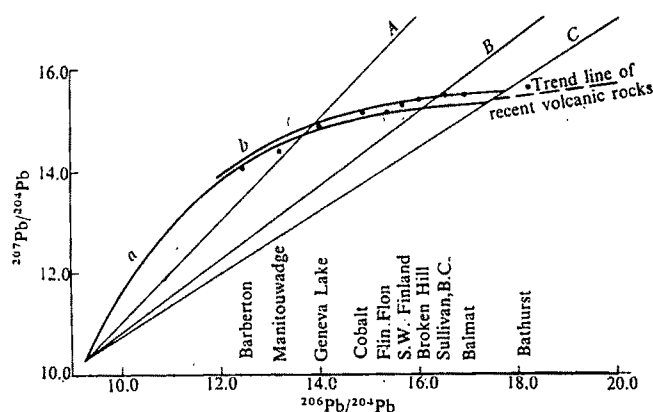


Fig. 1 Lead isotopic growth curves for $\mu = 7.8$ (a) and $\mu = 8.1$ (b), with isochrons for 2,500 m.y. (A), 1,000 m.y. (B) and 0 m.y. (C). The galena data from Table 1 are plotted, together with data from Sullivan, British Columbia, South-west Finland, and Cobalt, Ontario. See Russell¹⁰ for references to the original data. The trend line for recent volcanic rocks¹⁰ is shown for comparison.

that the source region for conformable galena deposits has experienced an increase in its U/Pb ratio at some time between 2,600 m.y. and 1,600 m.y. The data are plotted together with a few additional analyses in Fig. 1. Single stage growth curves for μ values of 7.8 and 8.1 are shown for reference. The general trend of the data suggests a parent system which has had a small, rather steady increase in U/Pb ratio with time. This may reflect an increasing component of lead from upper crustal sources in the younger galena deposits, or may represent evolution of the lower crust and upper mantle.

Two apparent paradoxes associated with the single stage growth curve have disappeared by using the new decay constants. Several authors have argued that the age of the Earth must be considerably greater than 4,550 m.y. since old galena and feldspar samples contained lead whose model age was older than the geological age of the specimen^{7,8,9}. Using the most recent decay constants and initial lead composition for the Earth produces no cases in which model lead ages are older than the geological age of the samples, when either 4,550 m.y. or 4,570 m.y. is used for the age of the Earth.

The second apparent paradox concerned the comparison of lead isotope data from recent volcanic rocks with the galena model growth curve. Russell¹⁰ discussed the problem in detail and constructed a mixing model to try to resolve the paradox. Figure 1 shows the trend line for recent volcanic rock leads in relation to the galena growth curve. Using the old decay constants to construct the growth curve produced an intersection of the trend line with the geochron ($t = 0$ isochron), which fell at a much lower model μ value than that of the galena growth curve. Thus, one had to postulate a rather different history for the source regions of the volcanic rocks and the galena deposits. Use of the new decay constants produces an intersection of the volcanic trend line with the geochron at a model μ value of about 7.9. The volcanic data are better represented by a band of constant width with the same slope as the trend line. This band would intersect the geochron at model μ values of 7.8 and 8.0, very close to the range in model μ values indicated by the galenas. We may therefore postulate that the source regions of the volcanic rocks and the galenas have had similar histories in terms of their lead isotopic development, although the highest model μ values for the youngest galenas might suggest that the fractionation of U and Pb in the galena source regions either began earlier, or was somewhat more intense than the fractionation in the source regions for the volcanic rocks.

Considering the rather drastic changes brought about by the use of the new decay constants and initial lead isotopic composition, it is perhaps prudent to consider the error limits

on the present set of parameters used in model age calculations. The main chance of error in the initial lead isotopic composition would be if the troilite nodule analysed by Tatsumoto *et al.*³ contained a small amount of radiogenic lead as well as primordial lead. Lowering the initial $^{206}\text{Pb}/^{204}\text{Pb}$ from 9.307 to 9.214, and the $^{207}\text{Pb}/^{204}\text{Pb}$ from 10.294 to 10.236 would result in a change in model age of only 13 m.y. It is extremely unlikely that the initial lead composition was as low as these hypothetical values. It is possible that the age of the Earth might be slightly greater than the value of 4,570 m.y. used in the calculations above—although there is no positive evidence that the Earth is older than the Moon and meteorites. If we increase the age of the Earth to 4,600 m.y., the Barberton model age is lowered by 40 m.y. to become 3,040 m.y. and the Balmat model age is lowered by 125 m.y. to 460 m.y. The uncertainty in the decay constants of uranium is claimed to be less than 0.1% (ref. 4). This introduces an uncertainty of ± 7 m.y. in the model age of the oldest samples and ± 20 m.y. in the youngest model ages. There seems to be no likely source of error which would lead to a significant bias towards young ages among the model ages calculated with the new parameters. Pending availability of more data on meteorite ages, it is suggested that uncertainties in the model ages of old samples be assigned at between +10 and -40 m.y. and for young samples at between +20 and -125 m.y.

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Post-Cretaceous faulting in Sudan and its relationship to the East African Rift system

It has been widely believed that no relatively young faulting has taken place in Sudan since the Mesozoic. The national Geological Map of Sudan, scale 1:4 million, 1963 edition, and the International Tectonic Map of Africa, scale 1:5 million, 1968 edition, show no faults in the territory and indeed the most recent comprehensive reviews of the structure of Sudan^{1,2} indicate that the late Phanerozoic formations are not significantly affected by faults.

In northern Sudan the Nubian Sandstone Formation is the most extensive of the Phanerozoic strata. It is of Cretaceous age and comprises basal conglomerates, thick cross-bedded sandstones and mudstones. It has been stated¹ that no major faults affect this formation but that minor fractures with a displacement of only a few feet occur near Wadi Halfa and that minor faults affect sedimentary strata in the Red Sea Hills in the east of the country.

Recent geological mapping, geophysical investigations, and aerial photography and satellite imagery in various places across central and northern Sudan have indicated considerable faulting with significant downthrows. The faults can be recognised on the ground by stratigraphic dislocation, brecciation, iron mineralisation and silicification. The latter is highly developed along joint planes in the Nubian sandstones and both may be genetically related.

Evidence of faulting of the Nubian Sandstone Formation has been observed at several localities (Fig. 1), which can be summarised as follows.

(1) South of Khartoum. Along the White Nile valley older E-W and younger N-S to NNW faults with downthrows normally to E and NE occur, and at Jebel Aulia a NNW-trending fault has a displacement of more than 1,200 m^{3,4}. The partial effect of faulting and warping has been the Gezira depression, in which buried Nubian Sandstone Formation is preserved beneath Tertiary to Pleistocene sediments (Gezira Formation) up to 110 m thick.

(2) North of Khartoum. The sedimentary cover to the Sabaloka basement inlier is displaced by E-W and N-S faults^{5,6}, with probable rejuvenation along earlier planes of weakness in the basement.

(3) Central Sudan, Kordofan Province. At Bara village (Fig. 1) a NW-SE trough was revealed by gravity measurements and drilling^{7,8}. The depression is 50 km wide, at least 150 km long, and more than 400 m deep. It is filled with Nubian Sandstone Formation and Tertiary to younger fluvial sediments (Umm Ruwaba Formation; compare Gezira Formation).

(4) En Nahud to Fula, Kordofan Province. Geophysical work⁹ supported by drilling revealed faulted Nubian Sandstone Formation troughs trending NNW at Fula and probably forming a large NW-SE basin beneath Babanusa. Along the south edge of the sediment-filled Nahud outlier¹⁰ E-W faulting was indicated.

(5) Western Sudan, Darfur Province. Fault-controlled NW-SE depressions¹¹ near El Fasher (Fig. 1) contain more than 1,200 m of Nubian Sandstone Formation. Near Zalingei a broad zone of fractures displacing basement gneisses and lower Palaeozoic strata strikes NNW to NW and is associated with Tertiary to Recent volcanism¹². The zone is covered by recent sediments south of Babanusa.

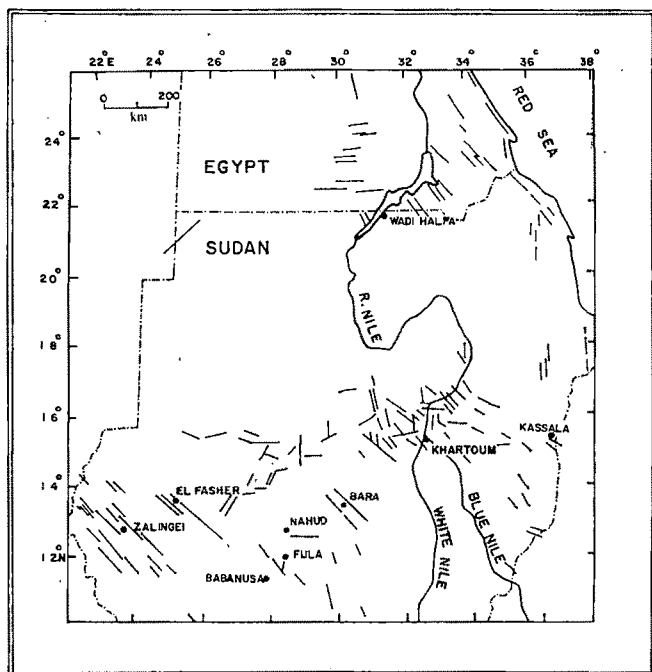


FIG. 1 Sketch map to show the distribution of post-Cretaceous faulting in Sudan and southern Egypt. Modified after Said¹⁸ and Vail²⁶.

(6) Eastern Sudan, Kassala Province. Faults displacing Nubian Sandstone Formation type sediments, the Gedaref Sandstone¹ and Tertiary basalts are recorded¹⁸⁻¹⁶ with general NW and NNE trends and with downthrows of up to 120 m.

(7) Northern Sudan and southern Egypt. Near Wadi Halfa faults trend E-W, NW or NNE¹⁷⁻²⁰. In Egypt faults trending N-S and NNW extend over 20 km with displacements up to 50 m (ref. 17). West of the Nile (Fig. 1) major faults cut Nubian Sandstone Formation and Upper Cretaceous strata; displacements are unknown.

(8) Red Sea coast. Tertiary sedimentary rocks along the coastal strip are affected by NNW, ENE and N-S faults^{18,21} with downthrow usually exceeding 50 m; total displacements may be considerable. Inland, faulting is difficult to recognise in the basement but satellite imagery indicates N-S to NW fracture patterns parallel to the Red Sea coast.

The age of the faulting may be indicated from the rocks affected. Across most of the northern half of Sudan the faults cut the Nubian Sandstone Formation of Cretaceous age, whereas in parts of eastern and western Sudan the faults displace Tertiary basalts. In southern Egypt E-W faults displace Nubian, Upper Cretaceous and lower Tertiary strata¹⁸. It may be assumed that most of the movement occurred during early Tertiary to more recent times. In Kordofan Province, for example, igneous activity of alkaline and carbonatitic character²² is apparently related to a fracture zone in which the most recent movement was only a few years ago²³.

The relationship between the post-Nubian faulting in the Sudan and the East African rift system is not known at present. The Egyptian geologists^{17,18,24} tend to consider a number of troughs or rifts to be present; however, there is some doubt whether these fractures can be directly related to rift valley tectonics²⁵ as has been claimed^{19,24}. Several of the fault depressions such as those at Jebel Aulia (just south of Khartoum), Bara and El Fasher are of the same order of magnitude as parts of the rift valleys of East Africa, and some have compatible trends. Several have been postulated as being structural features of the East African system^{7,18,19,24}. Unlike the East African rifts, however, the surface expression in Sudan is negligible, the grabens being for the most part filled by more recent sedimentary deposits, and buried beneath a superficial cover. The faulting has been assumed to have been mainly of Tertiary or Quaternary age and in this respect it is comparable to the main periods of rift faulting during the Miocene further to the south.

It seems therefore that the post-Nubian faulting in Sudan is broadly contemporaneous with the East African rift system, although morphologically not so evident at the surface, and that it probably resulted from related tectonic activity and associated volcanism that produced the East African system. Until more detailed mapping has been done, however, these fractures in Sudan should certainly not be referred to as rift valleys.

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BIOLOGICAL SCIENCES

5-Hydroxytryptamine accumulation in cerebrovascular injury

SEVERAL reports¹⁻³ indicate that some biogenic amines may significantly affect the intensity of the brain tissue reaction to injury, and that 5-hydroxytryptamine may be an essential factor in the production of brain oedema. This study was undertaken to explore this possibility, using as an experimental model brain oedema produced by the application of a cold metal plate to the exposed cerebral cortex of the cat⁴. This procedure results in severe damage to the upper cortical layers, including the disruption of blood vessels and the development of a vasogenic type of oedema. Vasogenic oedema is characterised by extracellular oedema fluid, containing extravasated serum constituents, spreading preferentially through the underlying and adjacent white matter⁵. It has been shown that the leakage of serum constituents is confined to the site of cortical injury, and that vascular permeability in the area of oedematous white matter itself, as tested for by protein tracers, remains unchanged⁶.

In the course of our investigations, we discovered that 5-hydroxytryptamine accumulates to a striking degree in the blood vessels in the region of cortical injury, although the oedematous white matter itself does not show any appreciable change in its 5-hydroxytryptamine levels. Here we describe this previously undescribed manifestation of nervous tissue reaction to severe injury.

Cold lesions were produced in cats by a procedure described previously⁴, and the animals were killed after 6 h. Corionally sectioned blocks of the brain, 2 mm in thickness and each containing the area of cold injury and adjacent gyri, were subjected to freeze-drying and processing according to the Falck technique⁷. Under the fluorescence microscope, the blood vessels within the area of cold injury showed a bright, yellowish-green fluorescence. Fluorescence of this nature was conspicuous in the walls of arteries, veins, and capillaries (Fig. 1). A few blood vessels showed an irregular, 'mottled' pattern of yellowish-green fluorescence in their lumina. Otherwise, the thrombotic material filling numerous blood vessels revealed only a faint bluish autofluorescence.



FIG. 1 Area of cortical cold injury, with blood vessels exhibiting a bright, yellowish-green fluorescence in their walls.

The characteristic yellowish-green fluorescence was not observed either in brain tissue adjacent to the fluorescent vessels or in blood vessels outside of the region of injury.

Microspectrofluorometry of the yellowish-green fluorescent vessels in the tissue sections revealed that this fluorescence had the excitation-emission spectra and photodecomposition rate characteristic of 5-hydroxytryptamine (Fig. 2).

The area of cortical cold injury and the underlying oedematous white matter were assayed for 5-hydroxytryptamine using a modification of the method of Maickel, *et al.*⁸ 5-Hydroxytryptamine in the injured cortical region was $329 \pm 30\%$ (mean \pm s.e.m.) of that in the contralateral uninjured cortex; that in the underlying oedematous white matter remained at control levels.

Platelets seemed to be a potential source for the 5-hydroxytryptamine accumulating in the blood vessels. We tested this hypothesis by labelling platelet 5-hydroxytryptamine, producing cold lesions, and measuring the specific activity of 5-hydroxytryptamine in control and injured brain areas.

Whole cat blood (10 ml preserved with USP Formula A citrate dextrose) was incubated with ³H-5-hydroxytryptamine creatinine sulphate (specific activity 7.3 Ci mmole⁻¹), in a final concentration of 1.37×10^{-6} M, for 30 min at 37° C. Labelled blood was re-injected into the cat, and a lesion was made. Tissue 5-hydroxytryptamine was extracted into butanol and acidified heptane, after Maickel, *et al.*⁸ and counted by liquid scintillation. 5-Hydroxytryptamine in the injured cortex had a specific activity 1500% of that in the control cortex and of that in both oedematous and control white matter.

After intravenous injection of 100 μ Ci of ³H-5-hydroxytryptamine binosalate (specific activity 1.0 Ci mmole⁻¹), 5-hydroxytryptamine in the injured cortical region had a specific activity 800% of that in control cortex, oedematous white matter, and control white matter.

The data suggest that a major portion of the 5-hydroxytryptamine accumulating in the area of the cold lesion is contributed by platelets. In addition, preliminary observations on cortical cold lesions in other species indicate a correlation between platelet 5-hydroxytryptamine content and the presence of fluorescent vessels. Species known to have appreciable quantities of 5-hydroxytryptamine in their platelets, such as rabbit, exhibit fluorescent vessels in the area of injury; species whose platelets contain little 5-hydroxytryptamine, such as the rat, show no fluorescence of vessels.

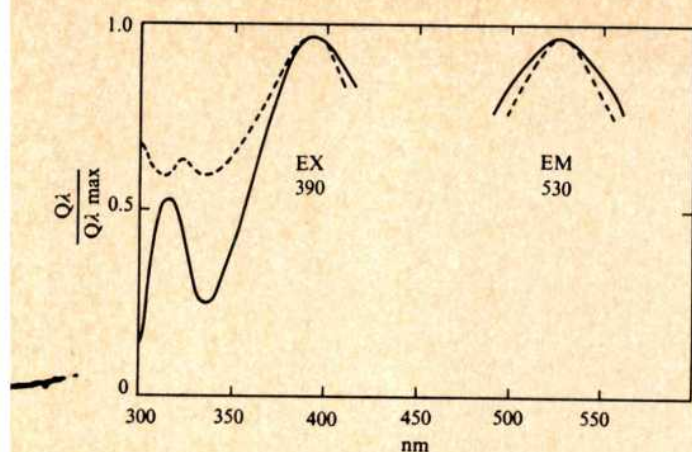


FIG. 2 Excitation (EX) and emission (EM) spectra of a fluorescent blood vessel (—), and of a 5-hydroxytryptamine oxalate sample (---) dissolved in buffered 1% gelatin at a concentration of 1 mg ml⁻¹. Microspectrofluorometry was carried out using a modified Leitz fluorescence microspectrophotograph. A quartz optical system was utilised for obtaining excitation spectra¹².

The exact structural localisation of the 5-hydroxytryptamine observed in blood vessels remains uncertain because of the limited resolution of the light microscope. The lack of fluorescent material in the lumina of many fluorescent vessels suggests that platelets entrapped in luminal thrombi have released their endogenous 5-hydroxytryptamine. In this context, it has been shown that platelet 5-hydroxytryptamine is rapidly released during thrombus formation^{9,10}. It seemed, in some instances, as if 5-hydroxytryptamine was localised in the endothelial lining or muscular coat of the vessels. Presumably, it is sequestered cytoplasmically in such a manner that it is not degraded rapidly by mitochondrial monoamine oxidase.

The significance of 5-hydroxytryptamine accumulation in injured blood vessels remains to be ascertained. It is possible that this phenomenon may occur in vascular injuries other than those produced by cold, probably in those associated with thrombus formation. The possibility that 5-hydroxytryptamine acting on cerebral blood vessels may affect their permeability is supported by the recent observation of Westergaard and Brightman¹¹ that 5-hydroxytryptamine and other biogenic amines reaching arterioles and venules from the brain parenchyma may alter their permeability to protein tracers. It is conceivable that the presence of appreciable amounts of 5-hydroxytryptamine in injured blood vessels may significantly affect the duration and intensity of vascular leakage, which is a crucial factor in the dynamics of vasogenic brain oedema.

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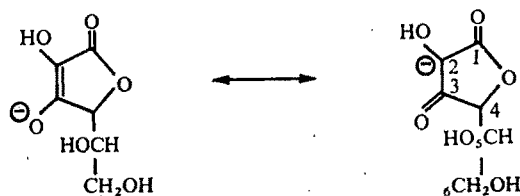
Ascorbic acid and biological alkylating agents

THE dangers to health from nitrite in the environment generally, and its use in meat processing in particular, have been pointed out by several groups¹⁻³. These dangers are associated with the production of nitrous acid in the acid environment of the stomach and the further reaction of this substance with naturally occurring and synthetic secondary and tertiary amines to form nitroso compounds which may be carcinogenic, mutagenic and cytotoxic. Ascorbic acid has been shown to effectively compete with amines for nitrous acid *in vitro*⁴ and it has been suggested that co-administration of ascorbic acid with potentially nitrosatable drugs, and foods containing nitrite, may reduce the hazards associated with ingestion of these substances⁴.

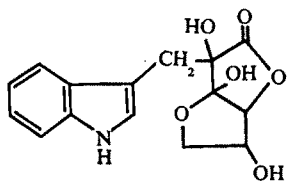
Kamm *et al.*⁵ have recently demonstrated the protective effect of ascorbic acid on the hepatotoxicity resulting from dual administration of sodium nitrite and aminopyrine to rats. Hepatotoxicity in this case is believed to be due to the formation of dimethylnitrosamine by reaction of nitrous acid with aminopyrine. They also reported⁵ that administration of sodium ascorbate, together with an oral dose of dimethylnitrosamine, partially prevented the liver damage produced by dimethylnitrosamine alone, thereby demonstrating an ascorbate-mediated protection which does not involve competition for nitrous acid. I wish to suggest a mechanism for the latter protective effect which may have general importance in the detoxification of environmental alkylating agents and in explaining certain aspects of their biological effects.

Many carcinogens, including dimethylnitrosamine, are believed to function as alkylating agents. They are either alkylating agents *per se* or they are metabolically converted into alkylating agents. A little-appreciated chemical property of ascorbic is its ability to react with alkylating agents⁶⁻⁹. At physiological pH, ascorbic acid (*pK*_a, 4.2) is largely in the anion form (I) and it is therefore particularly susceptible to alkylation *in vivo*¹⁰. The formation of ascorbigen (II) in plants¹¹⁻¹³ clearly illustrates the reactivity of ascorbic acid towards alkylating agents. Formation of ascorbigen involves alkylation of the ascorbic acid anion (I) by indole-3-methanol (III)⁸. This occurs very rapidly following the formation of indole-3-methanol in certain plant tissues in response to injury.

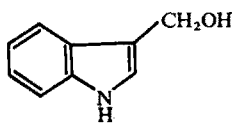
Under physiological conditions alkylation of the ascorbic acid anion (I) will occur mainly at C₂ of the ambident nucleophile to produce a new carbon-carbon bond, rather



(I)



(II)



(III)

than at the alternative oxygen site^{6,7} (compare ascorbigen). The carbon-carbon bond formed, unlike the carbon-oxygen bonds normally produced by the reaction of alkylating agents with other types of acid groups found *in vivo*, such as, carboxylic and phosphoric acids, will not be particularly susceptible to hydrolytic cleavage. Alkylation of ascorbic acid will therefore effectively compete with the alkylation of other cellular nucleophilic sites such as those on nucleic acids and proteins. It is this competition which I believe may provide a mechanism for the observed protective effect of sodium ascorbate on the hepatotoxicity of dimethylnitrosamine.

Ascorbic acid, if present in sufficient concentration, may also afford protection from the adverse effects of a number of other environmental alkylating agents by the same mechanism and the maintenance of high tissue levels of ascorbic acid may be of value for this reason. Some animal species, unlike man, are capable of synthesising ascorbic acid and this synthetic capacity, besides supplying the basic ascorbic acid requirement, may constitute a part of the metabolic defences of these animals. Thus a number of chemicals, including the carcinogenic hydrocarbons, 3,4-benzpyrene and 3-methylcholanthrene, are powerful stimulators of ascorbic acid synthesis in several animal species^{14,15}. The same chemicals are also potent stimulators of certain drug-metabolising enzymes in the liver¹⁵. Whereas the action of these enzymes detoxifies many harmful compounds, in some instances they convert chemicals to more toxic alkylating derivatives. The stimulation of ascorbic acid biosynthesis which accompanies the increase in activity of the drug-metabolising system may therefore represent an adaptation which, by providing a means of inactivating the alkylating metabolites produced by the drug-metabolising enzymes, would be of considerable benefit to the animal.

A extension of the above suggestion is that some of the biological effects of alkylating agents, which have in the past been mainly associated with their ability to alkylate nucleophilic sites on nucleic acids and proteins, may stem from the alkylation and rapid depletion of cellular ascorbic acid. It has been reported¹⁰ that a common feature of a number of carcinogens is their ability to degranulate the rough endoplasmic reticulum of cells¹⁶. Ascorbic acid deficiency in the fibroblasts of guinea pigs is characterised by a marked reduction in the extent of the rough endoplasmic reticulum, an increase in the number of free ribosomes and disorientation of the ribosomes which remain attached to the membrane^{17,18}. These abnormalities are reversed by

treatment with ascorbic acid, early changes being observed four hours after treatment with ascorbic acid and a return to an essentially normal state after 24 h^{17,18}. Thus ascorbic acid appears to be important for the normal attachment of polyribosomes to the endoplasmic reticulum and the degranulation of the rough endoplasmic reticulum associated with the action of carcinogens may, at least in some cases, be symptomatic of a rapid depletion of ascorbic acid.

Existing evidence supports the view that carcinogens will deplete animals of ascorbic acid. Smoking is reported to lead to a significant loss of ascorbic acid¹⁹. The carcinogen benzanthrone is known to markedly deplete guinea pigs of ascorbic acid²⁰ and some of the symptoms of benzanthrone toxicity have been successfully treated with ascorbic acid^{20,21}. Carbon tetrachloride, another carcinogen which is believed to act through a radical intermediate and which is known to degranulate the rough endoplasmic reticulum of hepatocytes¹⁶, provokes a marked depletion of ascorbic acid in the liver of rats²². Ross and Benditt¹⁷ have remarked on the similarity in appearance of the scorbutic fibroblast and the carbon tetrachloride damaged hepatocyte and ascorbic acid is reported to strongly protect guinea pig hepatocytes from carbon tetrachloride damage²³ suggesting that a rapid depletion of cellular ascorbic acid may play an important part in carbon tetrachloride toxicity.

There can be little doubt that a rapid depletion of cellular ascorbic acid will have an adverse effect on a number of cellular processes. The possibility that biological alkylating agents and some other chemicals such as carbon tetrachloride may be capable of this action should therefore be more widely known.

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Possible involvement of cyclic GMP in growth control of cultured mouse cells

NON-TRANSFORMED fibroblastic cells in tissue culture exist in one of two reversible growth states, a state of rapid proliferation (growing) and a state of relative quiescence (resting)^{1,2}. Transition from a resting to a growing state can be accomplished by a variety of mitogenic agents including growth substances in animal sera, insulin and proteases. 3', 5'-cyclic adenosine monophosphate (cyclic AMP) was implicated in this transition^{3,4} in that the intracellular concentrations of cyclic AMP in resting cell cultures fall after brief exposure to mitogenic agents^{3,4} and exogenous additions of high concentrations (10^{-4} to 10^{-3} M) of dibutyryl cyclic AMP to the culture medium cause a partial reversal of the mitogenic response⁵. Kram and Tomkins⁶ have shown that exogenous additions of 3', 5'-cyclic guanosine monophosphate (cyclic GMP) to the medium of cultured mouse cells can counteract the inhibitory effects of dibutyryl cyclic AMP upon some of the earliest events induced by a mitogenic signal (increased uptake of uridine, leucine, and 2-deoxyglucose). We now report (1) that relatively high concentrations (10^{-6} to 10^{-4} M) of cyclic GMP or its butyrate analogues when added to quiescent Balb/c 3T3 cultures can induce a substantial increase in DNA synthesis and (2) that within a few minutes of serum addition to quiescent cultures the intracellular concentration of cyclic GMP, as measured by two independent methods, rises by a factor of nine- to eleven-fold. Cyclic AMP and cyclic GMP concentrations were also measured throughout the remainder of the cell cycle.

Balb/c 3T3 cells obtained from Dr S. Aaronson were routinely grown in 10 ml of Dulbecco's modified Eagles medium (DEM) with 10% calf serum (Colorado Company) in 9 cm Petri dishes at 37°C under a 10% CO₂ (v/v) atmosphere⁷. For determinations of the intracellular concentrations of cyclic nucleotides monolayer cultures were quickly washed with Tris-buffered saline, 1 ml of 5% trichloroacetic acid (TCA) added per dish, and the contents immediately frozen on dry ice. Cultures were then stored frozen. Samples were thawed and centrifuged and the supernatant was extracted with ether and lyophilised to dryness⁸. The combined dry residues of ten Petridish cultures (1.5×10^7 to 2×10^7 cells) were dissolved in 1.5 ml of 0.1 M Tris-HCl (pH 8.5), 0.005 M MgCl₂ and half the samples were digested with 10 μ g ml⁻¹ 3', 5'-cyclic nucleotide phosphodiesterase to check the authenticity of the products assayed³. Both phosphodiesterase-treated and untreated samples were applied to 2 ml Dowex columns (Biorad AG 1-X8, 200-400 mesh, formate form) and the cyclic AMP- and cyclic GMP-containing fractions eluted by 2 N and 5 N formic acid respectively. Solutions were lyophilised to dryness and redissolved in 0.05 M

sodium acetate at either pH 4.0 or pH 6.2 for cyclic AMP- or cyclic GMP-containing fractions respectively. ³H-cyclic AMP and ³H-cyclic GMP were added after the initial thaw-

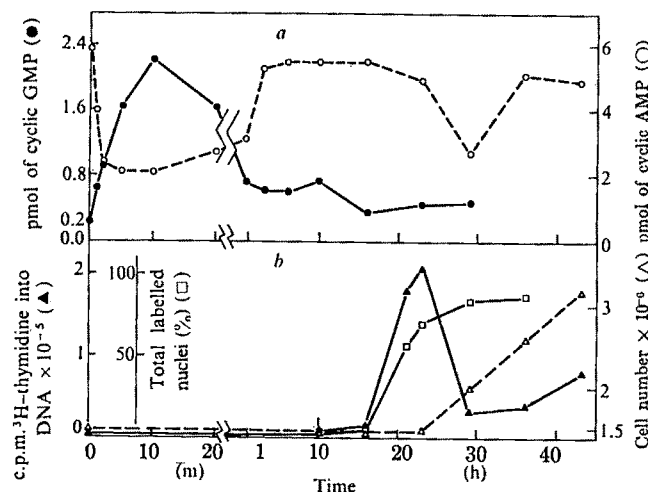


FIG. 1 Serum-induced sequential changes in cyclic nucleotide concentrations. *a*, Intracellular amounts of cyclic AMP⁹ (○) and cyclic GMP¹⁰ (●) were measured at different times after 20% serum and fresh medium addition to resting cultures of Balb/c 3T3 cells. Cell cultures were used 4 d after termination of DNA synthesis (less than 0.6% of the nuclei were labelled with ³H-thymidine in 18 h), and ten Petri dish cultures were isolated for each determination as described earlier. Similar results were observed if the Tris-buffered saline washing of the monolayer cultures was omitted. All results were corrected for differential losses during the isolation procedures (see earlier) and are expressed in pmol per 10⁶ cells. Duplicate results agreed to within 10%. Results after 3',5'-cyclic nucleotide phosphodiesterase digestion³ have been subtracted (0.1 and 0.3 pmol per 10⁶ cells for cyclic AMP and cyclic GMP respectively). Packed cell volumes and protein content per cell measured under these conditions do not vary more than 10% to 20% with changes in the cellular growth state¹², and thus the amounts measured reflect the relative intracellular concentrations. In the assays cyclic nucleotide amounts were measured in the range 0.1 to 1 pmol, and there was less than 1 part in 10,000 cross interference of cyclic AMP with the cyclic GMP radioimmuno assay. *b*, Parallel cultures were also radioactively labelled with 3 μ Ci ml⁻¹ ³H-thymidine at 3 μ M for 2 h and the c.p.m. of ³H-thymidine incorporated into DNA (Table 1) recorded for a fifth of the cultures; the time-point represents the midpoint of the 2 h pulse-label (▲). Other cultures were also continuously labelled with 3 μ Ci ml⁻¹ ³H-thymidine at 1 μ M from 5 h after medium change until the time recorded for isolation (□) and then processed for autoradiography⁷. The fraction of cells with radioactively labelled nuclei was recorded (□). Cell numbers per 9 cm Petri dish were estimated in a Coulter Counter as previously described¹² (Δ).

TABLE 1 Changes in DNA synthesis with exogenously-added nucleotides

Concentration (μM)	DB cyclic GMP	MB cyclic GMP	MB cyclic IMP	3', 5'-cyclic GMP	2', 3'-cyclic GMP	5'-GMP	DB cyclic AMP	cyclic AMP	cyclic CMP	MB cyclic UMP	cyclic UMP
2.5	10.2	21.6	5.7	5.8	-0.9	-0.7	0.6	1.2	0.7	1.2	1.1
25	20.3	22.5	28.4	5.0	0.2	-0.5	0.7	0.3	0.8	0.0	1.1
250	1.0	20.7	10.2	20.5	-1.0	-0.8	0.6	0.4	0.8	0.8	0.2

Results are expressed in thousands of c.p.m. of ³H-thymidine incorporated into DNA minus the value for control cultures with no additions (3,100 c.p.m.). Duplicate results agreed to within 15%. Abbreviations: DB, dibutyryl; MB, monobutyryl. Balb/c 3T3 cells were seeded in 5 cm Petri dishes at 10⁶ cells per dish in 5 ml of DEM medium containing 2% calf serum (v/v). Seven days later the medium was removed, cell monolayers washed twice with DEM alone, and fresh media containing 250 μ g ml⁻¹ bovine serum albumin, 0.5 μ g ml⁻¹ hydrocortisone (Calbiochem), and 0.1 ng ml⁻¹ of a purified pituitary growth factor¹¹ were added. After two further days (cell density 1.5×10^6 per dish) the various nucleotides (from Sigma and Boehringer) were added, and the cell cultures were radioactively labelled with 3 μ Ci ml⁻¹ methyl-³H-thymidine at 3 μ M from 12 to 30 h after nucleotide additions. Medium was then removed, cell monolayers washed twice with Tris-buffered saline and lysed with 2.0 ml of 0.5 N NaOH. One millilitre of the cell suspension was incubated 16 h at 37°C before the DNA was precipitated with 10% TCA and 50 μ g carrier calf thymus DNA. Precipitates were collected on glass-fibre filters and analysed for radioactivity¹². Addition of 20% calf serum yielded 112,000 c.p.m. of ³H-thymidine incorporated into DNA. Autoradiography⁷ of parallel cultures showed that approximately 1%, 15%, 18%, and 85% of the cell nuclei became radioactively labelled with ³H-thymidine after no addition, or the addition of 25 μ M DB cyclic GMP, 25 μ M MB cyclic GMP, or 20% serum respectively.

TABLE 2 Relative intracellular amounts of ^{32}P -cyclic nucleotides

	Resting cells	Serum-activated cells	Ratio: (Activated cells)/(Resting cells)
^{32}P -cyclic AMP (c.p.m.)	2,960	1,160	0.39
^{32}P -cyclic GMP (c.p.m.)	6,850	63,000	9.2
Labelled Nuclei (%)	0.8	83	100
DNA Synthesis (c.p.m.)	980	97,300	99

Forty Petri dishes (9 cm) of resting Balb/c 3T3 cells in DEM (2×10^4 cells per dish) were radioactively labelled with $100 \mu\text{Ci ml}^{-1}$ ^{32}P - H_2PO_4 for 11 h. Fresh medium containing 20% serum and $100 \mu\text{Ci ml}^{-1}$ ^{32}P - H_2PO_4 were added to twenty dishes (serum activated cultures) and all cells were isolated 19 min later as described. ^3H -cyclic AMP and ^3H -cyclic GMP were added to standardise the recovery and to act as reference markers. Approximately 1.5×10^4 c.p.m. of ^{32}P -TCA-soluble material was recovered in each case. After lyophilisation following ether extraction the dry residues were dissolved in 0.5 N HCl and chromatographed over successive cationic³ and anionic (Fig. 1) Dowex columns to remove ^{32}P - H_2PO_4 and nucleotide di- and triphosphates. Cyclic AMP- and cyclic GMP-containing fractions (Fig. 1) were lyophilised and dissolved in 0.1 M ammonium acetate (pH 7.0). Samples were applied to Whatman 3 MM paper and the chromatogram developed in a solvent of 3.5 parts of butanol, 2.5 of acetone, 4 of 0.5 M ammonium acetate (pH 7.5) for 38 h. The chromatogram was cut into 0.5 cm strips and analysed for radioactivity; the ^{32}P -cyclic nucleotides were well separated from other nucleotides (distances from origin: cyclic AMP, 40 cm; cyclic GMP, 33.8 cm; deoxy-TMP, 27.2 cm; AMP, 18.4 cm), and comigrated exactly with the ^3H -markers. ^{32}P -c.p.m. in the cyclic nucleotide regions were recorded for duplicate experiments which agreed to within 15%. Total cyclic nucleotide ^{32}P -c.p.m. were corrected both for losses in handling (approximately 50% of the ^3H markers were recovered) and for ^{32}P radioactive decay. Counts per minute of ^{32}P -ATP (or GTP) isolated from either resting or 20 min serum-stimulated cells were approximately the same, and thus the intracellular specific activity of ATP (or GTP) was constant. Comparison of ^{32}P c.p.m. of cyclic AMP and cyclic GMP do not reflect their relative concentrations in the cell as the intracellular specific activities of their precursor molecules (ATP, GTP) are different for a 12 h labelling period¹². Parallel cell cultures were also radioactively labelled with $3 \mu\text{Ci ml}^{-1}$ ^3H -thymidine at $3 \mu\text{M}$ from 14 to 28 h after medium change and the cultures isolated for autoradiography⁷ or DNA synthesis analysis (Table 1). Results are expressed as the fraction of cells with radioactively labelled nuclei (%) or as the c.p.m. of ^3H -thymidine incorporated into DNA for a tenth of one Petri dish culture.

ing of the cultures to monitor recovery, usually 70% to 80% of the input was recovered with less than 10% cross contamination in the respective cyclic nucleotide fractions. Cyclic AMP was measured by the protein kinase binding assay of Gilman⁹, and cyclic GMP by the radioimmune assay of Steiner *et al.*¹⁰.

Balb/c 3T3 cells could be maintained in a resting state in the absence of exogenously added serum by addition of very low concentrations of a growth factor purified from bovine pituitary glands¹¹ (less than 1% of the cellular nuclei became radioactively labelled with ^3H -thymidine in 18 h). Various nucleotides and their butyrate analogues were then added and Table 1 shows the changes observed in the incorporation of ^3H -thymidine into DNA. Only 3', 5' cyclic GMP and its butyrate analogues together with monobutyryl cyclic inosine monophosphate (cyclic IMP) yielded a substantial induction of DNA synthesis (eight to ten times the value in resting cultures); other nucleotides tested including cyclic AMP and 2', 3' cyclic GMP failed to do so. Results obtained exactly as described in Table 1 on addition of 250 μM monobutyryl cyclic GMP; cyclic GMP; GMP; or no addition to resting cell cultures maintained for 16 h in DEM without growth factor and hydrocortisone were 16,040; 12,030; 2,680; or 2,150 c.p.m. respectively. Therefore, the purified growth factor and hydrocortisone were not essential for the cyclic GMP enhancement of DNA synthesis but were included only to maintain a constant number of viable cells over a period of several days¹¹. In their absence, cellular viability as measured by the number of attached cells rapidly decreased after 48 h, and hence the results in Table 1 with the hormones present were obtained with non-degenerating resting cell populations capable of complete growth activation after serum addition (Table 1). The kinetics of induction of DNA synthesis were identical for serum or monobutyryl cyclic GMP addition to resting cultures: increases in DNA synthesis started at 14 to 16 h after additions (not shown). Addition of monobutyryl cyclic GMP to quiescent cultures^{7,12} of Swiss 3T3-4A, Balb/c 3T3 mouse cells, or baby hamster kidney cells seeded in 1%, 2%, or 1% calf serum respectively also caused a two- to three-fold stimulation of ^3H -thymidine incorporation into DNA over control cultures.

As the addition of cyclic GMP caused a significant induction of DNA synthesis presumably a certain fraction of the cellular population was induced to leave the resting state and enter the growing state. We then measured the intracellular concentration of cyclic GMP using a radioimmune assay¹⁰ at different times after addition of fresh medium and

serum to resting cultures of Balb/c 3T3 cells. Figure 1 shows that in addition to the two- to three-fold fall^{3,4,8} in the concentration of cyclic AMP shortly after medium change the concentration of cyclic GMP rose by a factor of eight- to eleven-fold over the value in unstimulated cultures reaching a maximum after 10 min. Thereafter the cyclic GMP value gradually declined and remained constant (within a factor of 2), although the cyclic AMP concentration rose again after 1 to 3 h to reach its maximum value before DNA synthesis (Fig. 1). Similar results were obtained with the 3T3-4A mouse line of cells (not shown). We also confirmed the changes in the relative cyclic nucleotide concentrations by radioactively labelling cells with ^{32}P - H_2PO_4 and directly isolating the cyclic nucleotides by chromatographic procedures. Table 2 shows that 19 min after fresh medium and serum were added to resting cultures of Balb/c 3T3 cells the cyclic GMP concentration was increased nine-fold, while the cyclic AMP concentration dropped by a factor of 2½-fold.

Fast, transient increases in the intracellular concentrations of cyclic GMP have recently been reported in other culture systems after administration of a mitogenic or mitogenic-like signal to quiescent cell cultures (for example, phytohaemagglutinin and concanavalin A on human peripheral blood lymphocytes¹³; insulin on rat fat cells or liver slices¹⁴), whereas cyclic GMP itself has mitogenic action on rat thymic lymphoblasts¹⁵ and can reverse the dibutyryl cyclic AMP inactivation of the immune response in precursors of antibody-forming spleen cells¹⁶. Here we have shown in the same culture system both the capability of relatively high (non-physiological) concentrations (10^{-6} to 10^{-4} M) of cyclic GMP to stimulate DNA synthesis in a significant fraction of quiescent mouse fibroblasts and large transient increases (ten-fold) in the concentration of intracellular cyclic GMP within a few minutes of exposure to a mitogenic signal (fresh medium and serum). Other events associated with transition from a resting to a growing state (increased protein and RNA synthesis¹²) are also stimulated by monobutyryl cyclic GMP addition to quiescent cultures (unpublished results). Exogenous addition of cyclic GMP may exert its mitogenic action by direct interference with the putative cellular growth-controlling machinery or by other less direct mechanisms such as the activation of the intracellular cyclic AMP phosphodiesterase¹⁷ to reduce cyclic AMP concentrations in the cell. The findings reported here, however, pose the possibility that cyclic GMP may act as a positive signal¹³ while cyclic AMP acts as a negative signal^{3,4,8} in the cell for controlling the growth of cultured fibroblasts.

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Possibility of inborn defect in isovalericacidaemia involving altered enzyme specificity rather than total inactivity

TANAKA, Isselbacher and colleagues¹ have described an inherited metabolic defect, isovalericacidaemia, which, though not fatal in their patients, leads, if untreated, to mental retardation and episodes of convulsion. As the name implies, the condition is characterised by high levels of isovaleric acid in the plasma. During periods of remission, this compound, which arises from the breakdown of dietary leucine, is excreted as a glycine conjugate. From the fact that only isovaleric acid accumulates and not butyric acid (from fat oxidation) or isobutyric or α -methyl butyric acids (from valine and isoleucine breakdown) Tanaka *et al.*¹ have concluded that there must be a specific isovaleryl CoA dehydrogenase that is non-functional in isovalericacidaemia. It had been assumed previously² that straight and branched-chain fatty acyl CoA compounds alike were oxidised by the green (that is, short-chain) acyl CoA dehydrogenase (see Fig. 1).

Sidbury, Smith and Harlan³ described an inherited neonatal condition in two unrelated families, the "odour-of-sweaty-feet" syndrome, in which the blood and urine apparently contained large amounts of hexanoate and butyrate. Symptoms of lethargy were followed by twitching, convulsions and death

within 2-3 weeks. Sidbury *et al.*³ attributed the condition to a faulty green acyl CoA dehydrogenase. Recent reanalysis of the origin samples by Ando *et al.*⁴ suggests that the original identification of the fatty acids was incorrect and that these also were cases of isovalericacidaemia. Ando *et al.*⁴ stress the need for rigorous identification of the characteristic compounds in establishing the existence of a new metabolic lesion.

Tanaka *et al.*^{5,6} noted several striking similarities between the symptoms of isovalericacidaemia and those of Jamaican vomiting sickness. The latter condition, frequently fatal, is not caused by an inborn error of metabolism but by the ingestion of a toxin, hypoglycin A, present in unripe 'ackee' fruits.^{7,8} Severe hypoglycaemia is accompanied in this condition by vomiting, and leads to coma. The hypoglycaemia has been attributed to abolition of a glucose-sparing effect in the under-nourished subjects through inhibition of fatty acid oxidation. Von Holt *et al.*⁹ suggested that methylenecyclopropane acetyl CoA, produced by the catabolism of hypoglycin A, might be an inhibitor of the short-chain acyl CoA dehydrogenase. Posner and Raben¹⁰ observed that hypoglycin also inhibits the oxidation of leucine, and Tanaka *et al.*⁵ have now shown that injection of hypoglycin A leads to greatly raised levels of isovaleric acid in the blood of rats. This they adduce in support of their contention that isovaleryl CoA dehydrogenase is a separate enzyme. They maintain that the coma in Jamaican vomiting sickness probably results from high levels of isovaleric acid rather than the low levels of glucose.

The view of isovalericacidaemia as an inherited deficiency of a specific and separate isovaleryl CoA dehydrogenase has already found its way into several respected textbooks. Bearing in mind, however, the strictures of Ando *et al.*⁴ with regard to chemical rigour, one should perhaps also proceed with caution in the biochemical interpretation of inborn errors of metabolism once the relevant compounds have been characterised. It is not impossible that a separate dehydrogenase exists for isovaleryl CoA, but so far no attempt has been made to purify it and demonstrate directly (1) that it is not the same as butyryl CoA dehydrogenase and (2) that one enzyme is absent or defective and the other present in isovalericacidaemia. The hypothesis also totally fails to explain the origin of the severe hypoglycaemia produced by hypoglycin A. It seems necessary, therefore, to re-examine critically the experimental evidence for the separate existence of isovaleryl CoA dehydrogenase.

In cases of isovalericacidaemia, during acute episodes the levels of isovaleric acid rise to about 30 mg per 100 ml, 500 times the normal figure. Levels of the other branched-chain fatty acids are not correspondingly elevated. In leukocytes from these patients the rate of oxidation of leucine to carbon dioxide is depressed ten-fold as compared with normal subjects. Rates of oxidation of other branched-chain amino acids were not measured.

Investigating the effect of hypoglycin A and its metabolites on rats, Tanaka *et al.*⁵ found that 0.7 mM α -ketomethylenecyclopropyl propionic acid (KMCP), a transamination product, inhibited conversion of 2-¹⁴C-leucine to ¹⁴CO₂ in liver slices by 87% and led to accumulation of isovaleric acid and its glycine conjugate. Their combined concentration was increased 180-fold. In addition, however, 2.1 mM KMCP inhibited isoleucine oxidation by 20% and led to accumulation of α -methylbutyrate (increased twenty-fold). Hypoglycin A itself (1.4 mM) also inhibited oxidation of isoleucine, though less strongly than that of leucine. Only valine oxidation was unaffected by hypoglycin A at this concentration. Leucine (60 mg per 100 g) orally administered to rats, following injection of hypoglycin A (10 mg per 100 g), raised the plasma level of isovaleric acid nearly 200-fold over the uninhibited control. In the same leucine-loading experiment, however, the level of butyrate was also raised forty-fold by the inhibitor. Moreover, in a similar experiment with

for the existence of a separate enzyme.

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Dramatic early increase in uterine eosinophils after oestrogen administration

Two separate receptor systems for oestrogens, thought to be involved in independent mechanisms of oestrogen action, have been found to exist *in vitro* and *in vivo* in the rat uterus. These are the cytosol-nuclear receptor system and the eosinophil receptor system¹⁻³.

The cytosol-nuclear receptor system exists in the epithelial, stromal and muscular cells of the uterus². This receptor system is thought to be responsible, through a two-step mechanism⁴⁻⁶, for the genomic response, which involves an increased transcription of mRNA. The mRNA then codes for the synthesis of specific proteins and enzymes, a process which results in the true growth of the uterus. The genomic response is blocked by actinomycin D⁷⁻¹⁰, but is not counteracted by 11-oxygenated corticosteroids¹¹⁻¹⁴. Oestradiol-17 β has a much stronger affinity than oestriol for the cytosol-nuclear receptor system and accordingly oestradiol-17 β is the stronger oestrogen for the genomic response¹.

The eosinophil receptor system has been demonstrated *in vitro*^{1,15} and *in vivo*² in the uterine eosinophil leukocytes of the mature rat. The eosinophil receptors have a high affinity, a limited binding capacity and a great specificity for oestrogens, but not for compounds without oestrogenic activity^{1,16,17}. The uterine eosinophil receptor system is con-

sidered to be involved in some of the early oestrogenic responses in the uterus, such as water imbibition, increase in vascular permeability, histamine-releasing and oestrogen-priming effects^{1,18}. These effects are not blocked by actinomycin D¹⁹, but are diminished by conditions that produce uterine eosinopenia (such as 11-oxygenated corticosteroids and progesterone) and are enhanced under conditions that produce uterine eosinophilia (see ref. 18 for a review). Oestradiol-17 β and oestriol have similar affinities for the eosinophil receptors and accordingly, both hormones are strong oestrogens for the early oestrogenic responses¹.

The eosinophil leukocytes are not present in the uteri of immature or ovariectomised animals, and they are attracted to this organ under oestrogenic conditions^{16,20-24}. To understand the role and importance of the uterine eosinophils and the mechanism of eosinotaxis it is necessary to know the kinetics of the oestrogen-induced uterine eosinophilia at early times. Eosinophils have been reported to increase in the uterus 12 h after the parenteral administration of oestradiol benzoate to castrated mice or rats²⁵. The increase in uterine eosinophils at earlier times was, however, overlooked. Here we describe a dramatic increase in uterine eosinophils as early as 5 min after an intravenous injection of oestradiol to immature rats.

Female immature Wistar rats, weighing 50 g, were used in the present experiment. The solution of oestradiol-17 β in saline-ethanol was injected under ether anaesthesia in the jugular vein, using a dosage of 30 μ g per 100 g body weight. The control rats were similarly injected with equal amounts of saline-ethanol. The animals were killed at various times after the injection, and the uteri excised. The right uterine horn was used for biochemical studies and the left uterine horn was fixed in neutral formalin for subsequent histological studies.

The following parameters were measured for each animal: uterine wet weight, DNA²⁶, RNA²⁷ and protein²⁸ content, eosinophils per uterine section (counted on thirty to forty sections) and total number of uterine eosinophils. The latter was estimated by multiplying the average number of eosinophils per section by an appropriate factor, taking into account the thickness of the sections and the total length of the fixed uterine horn.

There is a dramatic increase in uterine eosinophils, clearly evident as early as 5 min after the intravenous injection of oestradiol to immature rats (Fig. 1). At this time, the total number of uterine eosinophils have changed from less than ninety in the controls to 1,150 in the treated animals. The count of eosinophils per section, as well as the total number of uterine eosinophils increases continuously until 12-24 h (Fig. 1), when the total number stabilises at about 150,000. After 24 h, the number of uterine eosinophils diminishes progressively.

The increase in the uterine wet weight first becomes evident 3 h after the oestrogen injection. Increases in the RNA and protein content first appear 6 h after oestradiol injection. None of the above responses are apparent 1 h after oestrogen injection (Fig. 1).

Examination of the histological material also shows that a large number of uterine eosinophils are found, at early times, attached to the wall of the uterine capillaries and small veins. The eosinophils first appear in the uterus in the mesometrial pole of the uterine horn, especially between the muscular layers and in the mesometrium itself. At later times, they are distributed homogeneously throughout the uterus, as previously described¹⁵.

The very early increase in uterine eosinophils after oestrogen administration is of striking importance in the elucidation of the role of eosinophils in the early oestrogenic response. The eosinophils began to appear in the uterus within minutes, that is, earlier than several other changes in the early parameters of oestrogen stimulation, such as the water

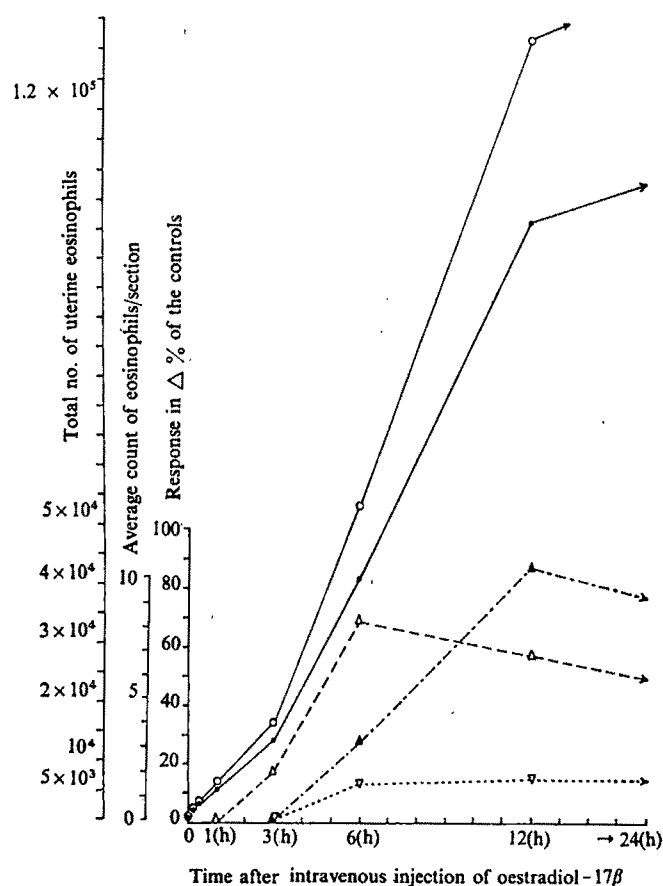


Fig. 1 Kinetics of oestrogen-induced uterine eosinophilia and other parameters of oestrogen stimulation. O, Total no. of uterine eosinophils; ●, average count of uterine eosinophils per section; Δ, uterine wet weight/DNA; ▲, RNA/DNA; ▽, proteins/DNA.

imbibition, histamine release and increase in RNA synthesis. An increase in cyclic AMP concentration is the only change reported at this very early time²⁹. Further data comparing the time sequences of early changes in cyclic AMP with the number of eosinophils in oestrogen-treated uteri are needed to discover if (and how) both parameters are related. The increase in cyclic AMP concentration has, however, been reported to be a transitory response²⁹, suggesting at least that the arrival of eosinophils in the uterus is independent of the continuous presence of cyclic AMP.

The rate of increase in the number of eosinophils in the present experiments suggests that the limiting factor of the oestrogen-induced uterine eosinophilia at early times, with the dose used in the present experiments, is the level of the eosinophils in the blood, which is low in the immature rat, and the production rate of eosinophils in the bone marrow.

The very early increase in uterine eosinophils after oestrogen administration suggests, although not conclusively, that eosinophils are directly attracted to the uterus by oestrogens. Previous *in vitro* and *in vivo* studies have demonstrated oestrogen binding by the uterine eosinophils^{1,2,15-17}. Autoradiographic studies of ³H-oestradiol *in vivo* have shown in eosinophils, besides the cytoplasmic localisation, the frequent appearance of silver grains along cytoplasmic interphases². This suggests the presence of a receptor for oestrogens near the cytoplasmic membrane of the eosinophils. *In vivo* studies have also demonstrated a higher concentration of labelled oestradiol along the walls of capillaries and small blood vessels in the rat uterus (unpublished data). The simultaneous presence of oestrogen receptors in the cytoplasmic membrane of the eosinophils and in the wall of the uterine capillaries and small blood vessels could be the key of the

organ-specificity of the oestrogen-induced uterine eosinophilia. It is possible that the oestrogens bound to the cytoplasmic membrane of the eosinophils also have affinity for receptors in the uterine capillary walls, thus allowing the attachment of the eosinophils to the capillary wall, which is the initial step towards penetration of the eosinophils into the uterus. The binding of oestrogens to the capillary wall may also induce conformational changes in its membrane which induces a specific attachment of the eosinophils to the capillaries. These hypotheses are under study.

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2-Phenylethylamine as a possible mediator for Δ^9 -tetrahydrocannabinol-induced stimulation

If neuroamines modulate perception and affect, as implied in much of the literature, the behavioural effects of marihuana may be mediated by alterations of brain amine mechanisms. Even in large doses ($5\text{--}500\text{ mg kg}^{-1}$), Δ^9 -tetrahydrocannabinol (Δ^9 -THC) produces only relatively small changes in the brain levels and turnover of serotonin¹⁻³, catecholamines²⁻⁴ and acetylcholine⁵. We report here biochemical and behavioural experiments suggesting a role for endogenous 2-phenylethylamine (PEA) in the action of Δ^9 -THC. PEA may be one of the neuromodulators involved in wakefulness, arousal and excitement^{6,7}, and it is the precursor of phenylethanolamine, a putative neurotransmitter⁸. PEA is present in human⁸ and animal⁹ brain; monoamine oxidase B plays a major role in the metabolism of PEA whereas monoamine oxidase A is responsible for the deamination of noradrenaline and serotonin¹⁰. The observations that the urinary excretion of PEA is decreased in patients with endogenous depression¹¹⁻¹³ and that in animals brain levels of PEA are increased by antidepressive treatments [monoamine oxidase inhibitors (MAOIs), imipramine, electroshock^{7,9,14}] suggest that this amine is important in modulating affect.

Brain levels of 2-phenylethylamine were determined in rabbits (New Zealand, male, 2-3 kg) using the method of Mosnaim and Inwang¹⁵, which measures the ultraviolet absorbance at 287 nm of the reaction product of PEA with ceric sulphate and HCl. For behavioural observation, ten male white Swiss mice (25 g) were grouped in a plastic cage [$28 \times 18 \times 12$ (height) cm]. Two observers used a simple checklist to evaluate behaviour in a 'blind' fashion. The number of experiments is listed in Tables 1 and 2.

Table 1 shows that 1 h after acute intravenous administration of Δ^9 -THC (3 mg kg^{-1}), brain levels of PEA had increased approximately four-fold. None of the other drugs studied in mice⁹, rats¹⁴, or rabbits¹⁶ induced such a large increase in PEA brain levels. Intravenous administration of 0.3 mg kg^{-1} of Δ^9 -THC (once daily for 8 d) doubled PEA brain levels (note that the psychoactive dose of Δ^9 -THC in humans varies from 0.01 to 0.25 mg kg^{-1} (ref. 17)). This increase in PEA brain levels may be due to a decrease in the rate of PEA disposition: in rabbits, Δ^9 -THC increased the amount of labelled PEA recovered from brain tissue following the intraventricular administration of trace amounts of labelled L-phenylalanine or of labelled PEA¹⁸.

Rabbits treated with Δ^9 -THC (0.3 or 3 mg kg^{-1}) showed a transient phase of excitement, followed by catalepsy with hyperexcitability to auditory stimuli (startle response was evoked by a soft clapping which evoked only an alerting response in untreated rabbits). Pargyline (100 mg kg^{-1} , given intraperitoneally, 72, 48 and 24 h earlier) increased the initial excitatory effect of Δ^9 -THC.

In mice, Δ^9 -THC (5 mg kg^{-1}) reduced spontaneous motor activity, and induced hyperexcitability and episodes of postural arrest with staring. In contrast, when mice were pretreated with the MAOIs nialamide (Table 2) or pargyline (100 mg kg^{-1} , 48 and 2 h earlier; or 100 mg kg^{-1} , 72, 48 and 24 h earlier), Δ^9 -THC increased motor activity and induced hyperexcitability and episodes of stereotyped behaviour ('popcorn effect') during which all the mice in the cage jumped, ran and vocalised. These episodes (lasting seconds or minutes) were separated by periods of relative quiet and occurred either spontaneously or were evoked by auditory stimuli. In the doses used, nialamide and pargyline inhibit brain MAO, and pargyline increases the levels of endogenous brain PEA¹⁹. Thus, the ability of these MAOIs to unmask this behavioural stimulant effect of

TABLE 1 Increase in brain levels of PEA induced by acute or chronic intravenous administration of Δ^9 -THC dissolved in 1 ml of Tween 80.

Treatment	mg kg ⁻¹	Time between injection and death (h)	Brain PEA mg kg ⁻¹ wet tissue $\bar{X} \pm \text{s.e.}$	No. of groups
None	—	—	0.42 ± 0.17	6
Tween 80	—	1	0.58 ± 0.20	6
Δ^9 -THC	3	1	$2.30 \pm 0.76^*$	6
Tween 80	Once daily, 8 d	24	0.66 ± 0.23	6
Δ^9 -THC	0.3 Once daily, 8 d	24	$1.36 \pm 0.36^*$	6

Each group consisted of six male white New Zealand rabbits (2-3 kg).

* $P = 0.01$ (treatment compared with control).

Δ^9 -THC suggests that the latter is mediated by endogenous monoamines. Of the monoamines that may mediate the stimulatory effects of Δ^9 -THC, serotonin is an unlikely candidate because its behavioural effects are mainly depressant¹⁹⁻²¹; moreover, the serotonin depletor *p*-chlorophenylalanine (Table 2) enhances rather than prevents the stimulatory effect of Δ^9 -THC (after MAOI) (see also related observations in refs 22 and 23). Endogenous catecholamines seem to inhibit rather than to mediate the stimulating effect of Δ^9 -THC since the catecholamine depletor α -methyl-*p*-tyrosine unmasks or enhances the stereotyped and aggressive behaviour induced by Δ^9 -THC in mice (Table 2). Our results suggest that PEA mediates Δ^9 -THC-induced stimulation. PEA readily crosses the blood-brain barrier and its amphetamine-like behavioural effects are potentiated by MAOIs^{7,9}. As in the case of Δ^9 -THC, the administration of

TABLE 2 Effect of Δ^9 -THC followed by PEA on grouped mice

Pretreatments	Treatments	Behaviour 'Popcorn effect'	Fighting	Activity
None	Solvent	0	0	—
	Δ^9 -THC	0	0	—
	PEA	0	0	—
	Δ^9 -THC + PEA	0	0	—
Nialamide (150 mg kg ⁻¹ , 48, 24 and 2 h earlier)	Solvent	0	0	—
	Δ^9 -THC	++	0	++
	PEA	+	0	+++
	Δ^9 -THC + PEA	+++	+++	+++
α -methyl- <i>p</i> -tyrosine (100 mg kg ⁻¹ 3 h earlier)	Solvent	0	0	—
	Δ^9 -THC	++	0	—
α -methyl- <i>p</i> -tyrosine (100 mg kg ⁻¹ 3 h earlier) + Nialamide	Solvent	0	0	0
	Δ^9 -THC	+++	+	++
DL- <i>p</i> -Chlorophenylalanine (150 mg kg ⁻¹ 72, 48, and 24 h earlier)	Solvent	0	0	—
	Δ^9 -THC	0	0	—
DL- <i>p</i> -Chlorophenylalanine (150 mg kg ⁻¹ 72, 48, and 24 h earlier) + nialamide	Solvent	0	0	+
	Δ^9 -THC	++	+	—

Δ^9 -THC was given in a dose of 5 mg kg^{-1} and was followed 1 h later by 6 mg kg^{-1} PEA. Mice were in groups of ten and each experiment was performed at least twice. All drugs were injected intraperitoneally (0.01 ml g^{-1} of body weight) and dissolved in saline; Δ^9 -THC was dissolved in saline and Tween 80 (24:1 (v/v)). Controls were injected with drug vehicles; saline controls were not different from untreated animals and are omitted from the table. Behaviour was evaluated by two observers in a blind fashion using a simple rating scale. The 'popcorn effect' is described in the text.

PEA (10 to 50 mg kg⁻¹) alone (without MAOI pretreatment) induced depression preceded by a brief stimulation, whereas in mice pretreated with MAOIs (doses as described above), PEA induced amphetamine-like stimulation, 'popcorn' behaviour and, less frequently, aggressiveness. PEA (6 mg kg⁻¹) did not significantly alter the behaviour of mice not pretreated with MAOIs and, in those that were pretreated it induced hyperactivity and a weak and brief 'popcorn' effect. Moreover, the stimulant effects of Δ^9 -THC and PEA are synergistic. In mice pretreated with nialamide (Table 2), the combination of Δ^9 -THC (5 mg kg⁻¹) and PEA (6 mg kg⁻¹, 1 h after Δ^9 -THC) induced marked hyperactivity, 'popcorn' behaviour and vicious fighting (defensive and aggressive postures, chasing, and biting to the point of drawing blood). A similar interaction between Δ^9 -THC and PEA was obtained in mice treated with pargyline except that fighting was not observed.

These behavioural experiments suggest that the observed increase in the brain levels of PEA induced by Δ^9 -THC are associated with an increase in free PEA available to receptor sites and that this is responsible for some of the stimulant effects of Δ^9 -THC.

These results were presented in part at the second international symposium on drug addiction in New Orleans, March 1973. We thank Mr Sam Myles for technical assistance. Drugs were supplied by the National Institute of Mental Health (Δ^9 -THC), Abbott Laboratories (pargyline), Pfizer Laboratories (nialamide) and Merck Sharp and Dohme (α -methyl dopa).

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Regeneration of the ventricular myocardium in amphibians

REGENERATIVE capacity of the myocardium in vertebrates is reported to be minimal¹⁻⁶. Mauro has advanced the thesis that the absence of satellite cells in the cardiac muscle is responsible⁷. Most recently, Oberpriller and Oberpriller have reported mitosis in adult newt myocardial cells 16 d after minor trauma⁸. Here we report observations on the adult salamander which indicate that cardiac regeneration of great competency occurs in this animal.

The adult aquatic stage of *Triturus viridescens* was used, animals were stored in pond water in glass aquaria at room temperature and were fed chopped liver and beef twice weekly. Anaesthesia was obtained with 1:1,000 Tricaine (Ayerst) and the heart was approached through a midline ventral incision, splitting the sternum and incising the pericardial sac. Between 30% and 50% of the ventricular myocardium was removed with iridectomy scissors traversing across the ventricular cavity (Fig. 1) and the skin incision was closed with interrupted 4/0 silk sutures. The animals were immediately transferred to fresh pond water in finger bowls and after recovery, returned to glass aquaria. No antibiotics or other chemical agents were used. During recovery, skin capillaries were observed microscopically in the tail fin, using transmitted light. For histological examination, the heart was removed *in toto*, fixed in buffered formalin with subsequent routine processing in hematoxylin and eosin. For electron microscopy, the hearts were fixed in cold 4% phosphate buffered glutaraldehyde, post-fixed in osmium tetroxide and embedded in Epon. Thin sections showing silver-grey interference colours were stained with uranyl



FIG. 1 Longitudinal sections of triturus heart showing extent of ventricular resection. Resection partially completed, entering into ventricular lumen from the right, in direction of the arrow. Excess portions of the auricles have been trimmed away after fixation.

acetate and lead citrate and examined with an RCA EMU-2D electron microscope.

In an initial series of twenty animals, all survived the procedure for two weeks after which they were killed. Since then, several hundred animals have been used for various phases of the study with an overall mortality rate of 10%. Observation of the heart *in situ* by dissecting microscope immediately after ventricular resection revealed pronounced contracture of the myocardium at the resection site resulting in a decrease in the diameter of the opening into the ventricular lumen. Bleeding was noted to be initially brisk, decreasing rapidly and ceasing with clot formation at approximately 60 s.

Complete circulatory stasis occurred within 5 min of resection. Erythrocytes were always trapped in the observed capillaries and after a variable period of time (30–120 min), oscillatory movement of these cells could be seen, with no directional flow. Directional movement (in which pulsations as such were not visible) began generally at 90 to 120 min but once occurred as early as 1 h. Circulation was noticeably slower than normal and generally did not become normal until 3–5 h after resection. Twelve animals were followed using smears (Giemsa-Jenner stained) of the peripheral blood at 30 min intervals following resection. At 2 h after resection, immature erythrocytes first began to appear and reached a maximum 15%–20% at 5 h.

Specimens for histological examination were taken at 5, 15, 30 and 60 min and 2, 3, 5 and 24 h after resection. Normal, non-operated specimens were processed at intervals to check on technique; no mitotic figures were observed in either the control or any of the experimental specimens. In the early experimental specimens, the major part of the clot was frequently lost in the process of removal and fixation of the heart. Many immature erythrocytes were visible, however, in the retained portions of the clot which were closest to the injured myocardium. After 30 min, the clot appeared to have differentiated into two zones, one closest to the injured myocardium which was composed of small basophilic cells with darkly staining nuclei and a peripheral zone of normal appearing erythrocytes with eosinophilic cytoplasm. After 3 h, the area of the resection was filled with a tightly packed mass of the basophilic cells (Fig. 2) and the original peripheral erythrocytes were undergoing

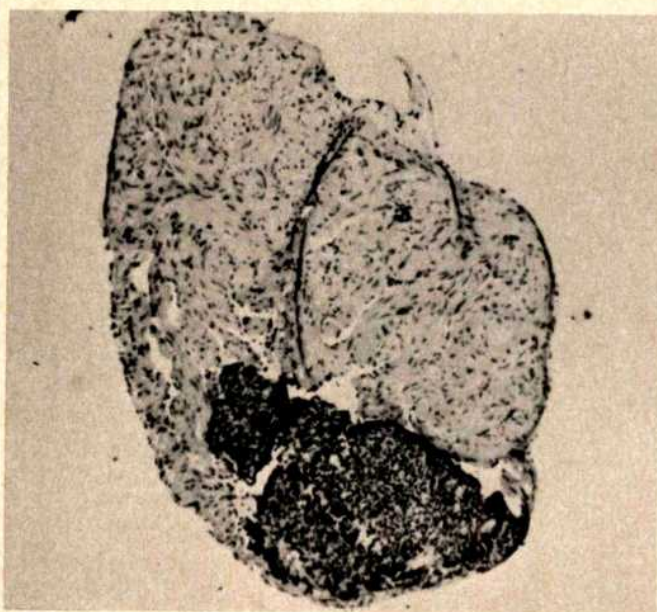


Fig. 2 Longitudinal section through ventricular myocardium 3 h after resection (magnification scale as Fig. 1). The mass of basophilic cells is clearly distinct, from the remainder of the myocardium and appears to be approximately the same in extent as the original resected portion.



Fig. 3 Electron micrographs through site of ventricular resection two hours after resection; section through one of the cells bordering the defect, showing a region of active myofilament synthesis. Many polyribosomal complexes (C) are seen in close association with filamentous structures. z-band material and associated myofibrils (Z) are seen in the early stages of organisation. Mitochondria (M) show densely packed cristae and an electron-dense matrix and the sarcoplasmic matrix has a plaque embryonal appearance.

degeneration and nucleolysis. Between 3 and 5 h, the mass of basophilic cells diminished and slightly basophilic myocardial fibres appeared, which were arranged in a more open 'lattice-like' fashion than the remainder of the myocardium. By 24 h, sections appeared completely normal and there was no evidence of injury other than, occasionally, a small mass of degenerating erythrocytes exterior to the myocardium proper.

Specimens for electron microscopy were taken on the same time scale as for histological examination (except at 24 h). The early stages, 5 to 30 min, primarily showed the young forms of erythrocytes previously noted. The myocardium at the edge of the defect demonstrated several signs of degeneration in the 30 min specimen. By 2 h, the cells closest to the myocardial edge showed active fibrogenesis (Fig. 3).

Unquestionably, these animals can survive resection of up to 50% of the ventricular myocardium, with restoration of normal circulatory dynamics within 5 h. It seems that this is accomplished by some active cellular process with restoration of the missing myocardium rather than simple approximation of the cut edges and subsequent 'sealing off'. The origin of the reparative cells and the mechanism used to restore the myocardial mass is, as yet, unclear. If this represents a true regenerative process, it is more rapid and competent than any previously reported.

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Thymine-labelled deoxyoligonucleotide involved in DNA chain growth in *Bacillus subtilis*

OKAZAKI *et al.* showed that bacteria labelled with short pulses of radioactive thymidine incorporate radioactivity into small DNA fragments, the so-called Okazaki pieces¹. These fragments have been observed in various bacterial, bacteriophage and eukaryotic systems^{2,3}. Pulse-chase experiments demonstrated that the Okazaki pieces are precursors of longer DNA chains, indistinguishable in length from the bulk of the DNA. Thus, the idea has arisen that DNA synthesis proceeds discontinuously on at least one of the two growing DNA chains of a given replication fork; that is, individual short DNA fragments are synthesised by a 5' → 3' DNA polymerase and then joined to the growing chain by ligase².

However, Werner has reported that cultures of *E. coli* labelled with short pulses of ³H-thymine incorporated radioactivity principally into large DNA; only a small fraction of the label was found in Okazaki pieces⁴. Furthermore, the fraction of the total label incorporated into Okazaki pieces increased rather than decreased with time of labelling during very short pulses. On the basis of these and other experiments, Werner concluded that DNA replication proceeds continuously without Okazaki pieces as intermediates⁴. Furthermore, he suggested that thymine and thymidine label separate precursor pools, thymine being used for replication and thymidine being incorporated primarily into Okazaki pieces involved in DNA repair⁵.

Using a thymine-requiring strain of *Bacillus subtilis*⁶, we decided to explore the possible differences between thymine (T) and thymidine (TdR) labelling of DNA. Cultures (80 ml) of *B. subtilis* *thy⁻ trp⁻* were grown in minimal medium at 25°C until late exponential phase (1.5×10^8 cells ml⁻¹), centrifuged and resuspended in 5 ml of fresh medium containing 5 µg ml⁻¹ T. The concentrated cells were incubated at 25°C for 10 min and then labelled with short pulses of ³H-T or ³H-TdR. Pulses were terminated with -20°C acetone, the cells lysed and the lysates sedimented in alkaline sucrose gradients.

Figure 1 compares the labelling patterns of cells pulsed with ³H-T against ³H-TdR. In both cases, most labelled DNA sediments slowly with an S value of 30 or less. Very little radioactivity is found at a position in the gradient corresponding to 75S, the sedimentation coefficient of bulk DNA under our conditions. (The radioactivity in the final fraction of each gradient is due to unlysed cells.) Figure 2 shows the labelling patterns found with longer pulses of ³H-T. Again, most labelled DNA sediments more slowly than bulk DNA. The S value increases gradually as the pulse time increases, in a manner similar to that reported by Okazaki *et al.* for ³H-TdR labelling of *B. subtilis*². The gradual transition shown in Fig. 2 is characteristic of *B. subtilis*; a significant number of intermediate size chains are observed, suggesting that joining of Okazaki pieces may be slower in *B. subtilis* than in *E. coli*².

Results similar to those seen in Figs 1 and 2 are also obtained by using unconcentrated cells, or by using a differ-

ent solution to stop the pulse (10% pyridine-20 mM KCN — 1 mM EDTA at 0°C)⁷. In all cases thymine labels

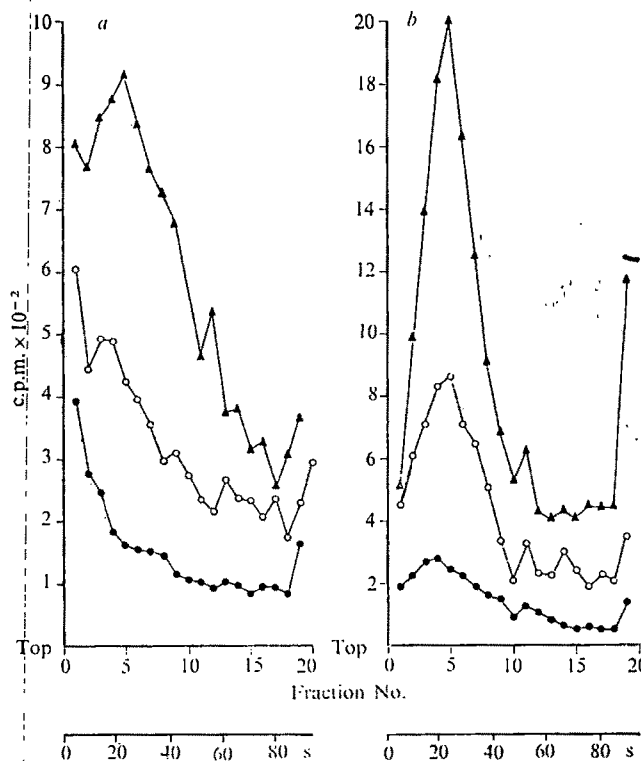


FIG. 1 Alkaline sucrose gradient sedimentation of pulse-labelled *B. subtilis* DNA. *B. subtilis* *thy⁻ trp⁻* was grown at 25°C in a medium containing 0.2% (NH₄)₂SO₄, 1.4% K₂HPO₄, 0.6% KH₂PO₄, 0.02% MgSO₄·7H₂O, 10⁻⁶ M FeSO₄·7H₂O, 10⁻⁷ M MnCl₂·4H₂O, 0.2% glucose, 0.2% Na₃ citrate·2H₂O, 0.2% casamino acids, 40 µg ml⁻¹ tryptophan, and 5 µg ml⁻¹ thymine. The doubling time is 110 min in this medium. For a single pulse labelling experiment, 80 ml of cells was grown to late exponential phase (1.5×10^8 cells ml⁻¹), collected by centrifugation at 4°C, and resuspended in 5 ml of fresh complete medium. The concentrated cells were shaken gently for 10 min at 25°C, then stirred vigorously with a magnetic stirrer, and pulse labelled with either 0.6 ml ³H-T (20 Ci mmol⁻¹, 1 mCi ml⁻¹) or 0.6 ml ³H-TdR (6 Ci mmol⁻¹, 0.1 mCi ml⁻¹). The pulse was terminated by the rapid addition of 20 ml of -20°C acetone. The cells were centrifuged at 4°C and then resuspended in 20 ml of a solution containing SSC (0.15 M NaCl, 0.015 M Na₃ citrate), 20mM KCN, 1mM EDTA. The cells were centrifuged again at 4°C and the cell pellet suspended in 0.15 ml of SSC containing 27% sucrose, 20 mM KCN, 10 mM EDTA, pH 8.2. A 25 µl sample of 40 mg ml⁻¹ lysozyme was added and the sample incubated for 30 min at 37°C. Then 0.05 ml of 10% sarkosyl and 0.1 ml of 1 M NaOH — 0.1 M EDTA were added. After gentle shaking, the cells began to lyse; 0.7 ml of 0.3 M NaOH and 30 mM EDTA were added slowly. The entire lysate was layered on top of a linear 5% to 20% (w/v) alkaline sucrose gradient containing 0.3 M NaOH, 0.5 M NaCl, 10 mM EDTA. Sedimentation was carried out in a Beckman L2-65B ultracentrifuge with an SW27 rotor spun at 20,500 r.p.m. for 16 h at 4°C. The centrifuge tubes were fractionated from the top with an Auto Densi-Flow fractionator (Buchler Instruments) into 19 fractions of equal volume. Individual fractions were precipitated by addition of 0.3 ml 0.1 M Na pyrophosphate, 0.3 ml 3 mg ml⁻¹ T, and 4 ml cold 15% TCA containing 0.50 mM Na pyrophosphate. The samples were kept at 0°C for 20 min, and the precipitates collected on nitrocellulose filters (Matheson-Higgins Co., 25 mm diameter, 0.45 µm pore size) which had previously been soaked in 50 mM Na pyrophosphate, 200 µg ml⁻¹ T. The filters were rinsed with cold 3% TCA, dried, suspended in a toluene-scintillator solution, and counted in a liquid scintillation counter. The S scale was determined using a Φ29 DNA marker (S = 26). a, cells pulsed with ³H-T; b, cells pulsed with ³H-TdR. Pulse time: ●, 8s; ○, 15s; ▲, 30s.

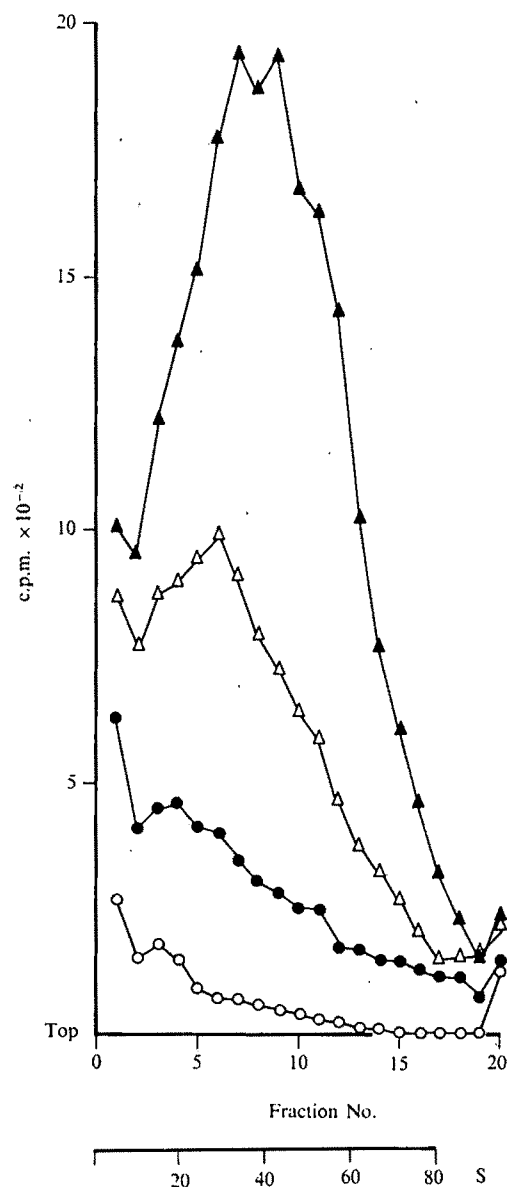


Fig. 2 Alkaline sucrose gradient sedimentation of pulse-labelled *B. subtilis* DNA ($^3\text{H-T}$). Conditions were as described in Fig. 1, except that $0.4 \text{ ml } ^3\text{H-T}$ ($12.6 \text{ Ci mmol}^{-1}$, 0.5 mCi ml^{-1}) was used for each sample. Pulse time: \circ , 15s; \bullet , 1 min; \triangle , 3 min; \blacktriangle , 6 min.

Okazaki pieces just as thymidine does, and the fraction of radioactivity in short DNA chains decreases rather than increases with the time of labelling. Pulse-chase experiments show that the thymine-labelled Okazaki pieces are precursors of larger DNA (data not shown). Independently, Okazaki *et al.* have shown that short pulses of $^3\text{H-T}$ are incorporated primarily into short DNA chains in both coliphage T4-infected cells and *E. coli*^{8,9}. Thus, there is no reason to doubt the common view that Okazaki pieces are intermediates in DNA replication, and that they can be labelled with either T or TdR.

We did observe one difference between T and TdR labelling of *B. subtilis*. In the case of $^3\text{H-T}$ labelling only, a significant amount of radioactivity can be seen in the top fraction (No. 1) of the sucrose gradients depicted in Figs 1 and 2. To separate the labelled material at the top of the gradient from Okazaki pieces, $^3\text{H-T}$ labelled lysates were sedimented for a longer time at a higher centrifuge speed. Figure 3 shows that a 15 s pulse of $^3\text{H-T}$ labels not only Okazaki pieces (fractions 9-14) but also a trichloroacetic acid (TCA)-precipitable component of about 2S (fractions 1-2). The 2S component is

labelled in very short pulses, before radioactivity appears in Okazaki pieces (Fig. 3).

We performed several control experiments to test if the rapidly labelled 2S component was actually a thymine-containing compound produced by live cells. First, we added $^3\text{H-T}$ to a non-radioactive lysate, centrifuged it through a sucrose gradient, and showed that there was no TCA-precipitable radioactivity in the gradient. Second, we noted that $^3\text{H-T}$, purified on a Sephadex G-25 column to separate it from potential macromolecular impurities, also could label the 2S component. Finally, we took fractions containing the 2S component from a sucrose gradient and treated them with formic acid, to hydrolyse the bases from the nucleic acids. Analysis of the hydrolysate by cellulose thin-layer chromatography demonstrated that the ^3H -labelled material co-chromatographed with a thymine marker, indicating that the 2S component is labelled with $^3\text{H-T}$ and not with a ^3H -labelled impurity.

To characterise the 2S component further, appropriate fractions were pooled from sucrose gradients, dialysed, and

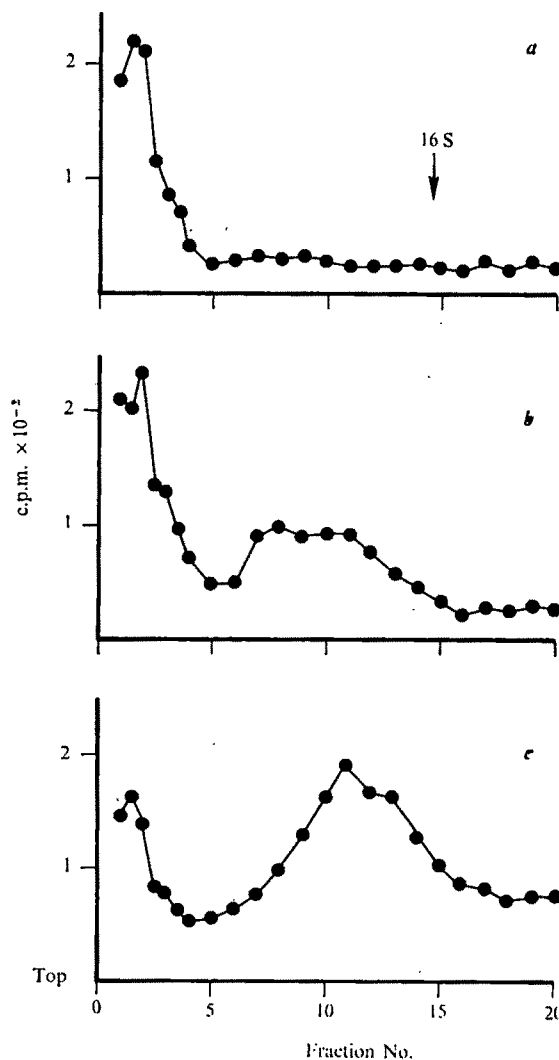


Fig. 3 Alkaline sucrose gradient sedimentation of pulse-labelled *B. subtilis* DNA. Conditions were as described in Fig. 1, except that $0.6 \text{ ml } ^3\text{H-T}$ ($12.5 \text{ Ci mmol}^{-1}$, 1 mCi ml^{-1}) was used for each sample, and sedimentation was carried out at 26,000 r.p.m. for 40 h. The 16S sedimentation marker, a gift of C. Mulder, was $^{32}\text{P-SV40}$ DNA converted to the linear form by the R_1 restriction enzyme¹⁵. The first six fractions collected from each gradient were half the usual volume, in order to improve the resolution. Therefore, the fraction indicated as No. 4 was actually the seventh collected. Fractions 4-20 were then collected in the usual manner. a, 3s; b, 8s; c, 15s.

subjected to treatment with various enzymes. In some cases Okazaki pieces were treated in a similar manner. Table 1 shows that the 2S component is degraded to TCA-soluble material by pancreatic DNase, snake venom phosphodiesterase, *E. coli* exonuclease I or hot TCA. However, it is resistant to RNase A, pronase or alkali digestion. This indicates that the 2S component is a deoxyoligonucleotide with a 3'-OH end. Furthermore, as Table 1 shows, the deoxyoligonucleotide is resistant to spleen phosphodiesterase, but becomes sensitive to spleen phosphodiesterase after digestion with bacterial alkaline phosphatase. Therefore, the 5' end of the deoxyoligonucleotide must be phosphorylated.

TABLE 1 Effect of various agents on 2S component

Agent	% degraded to TCA soluble	
	2S component	Okazaki pieces
Pancreatic DNase	83%	85%
RNase A	5%	—
0.3 N NaOH	5%	—
Pronase	15%	—
Hot TCA	90%	—
Exonuclease I	86%	—
Snake venom phosphodiesterase	80%	85%
Spleen phosphodiesterase	10%	6%
BAP	6%	9%
BAP + spleen phosphodiesterase	67%	50%

BAP, bacterial alkaline phosphatase.

The 2S component was obtained by pooling appropriate fractions from sucrose gradients similar to those shown in Fig. 3. Okazaki pieces were obtained from sucrose gradients similar to those shown in Fig. 1b. Samples were dialysed against either SSC (0.15 M NaCl, 15mM Na₃ citrate) or the solvents described below, and then treated as described below. Pancreatic DNase digestion, with 20 μ g ml⁻¹ enzyme, was performed at 37°C for 1 h in 10mM Tris, pH 7.6, 10mM MgCl₂. RNase A, heated at 80°C for 10 min at pH 5 in 0.15 M NaCl to inactivate contaminating DNases, was used at 50 μ g ml⁻¹, 37°C for 30 min. Pronase, self-digested for 1 h, was used at 1 mg ml⁻¹, 37°C, for 5 h. *E. coli* exonuclease I digestion was in a 1 ml reaction mixture containing 10mM MgCl₂, 1mM mercaptoethanol, 0.1 M glycine buffer, pH 9.4, and 35 U of enzyme, at 37°C for 1 h. Snake venom phosphodiesterase digestion was performed in 5mM MgCl₂, 10mM Tris, pH 8.0, with 160 μ g ml⁻¹ enzyme at 37°C for 1 h. Spleen phosphodiesterase digestion was in 0.3 M ammonium succinate, pH 6.5, with 2.5 U enzyme ml⁻¹ for 1 h at 37°C. BAP digestion was in 0.1 M Tris, pH 8.0, with 6 U BAP ml⁻¹ for 1 h at 37°C. When BAP and spleen phosphodiesterase were used together, the solvent was 0.3 M ammonium succinate, pH 6.5. The samples were incubated with BAP alone for 1 h, then spleen phosphodiesterase was added, and the incubation continued for an additional 1 h. Alkali treatment involved 0.3 M NaOH for 16 h at 37°C. Hot TCA treatment involved precipitating the sample in 5% cold TCA, and then heating it to 90°C for 30 min. In all cases, samples were precipitated with cold TCA, filtered, and assayed for radioactivity as described in the legend to Fig. 1.

In view of the importance of RNA priming for DNA synthesis^{10,11}, the deoxyoligonucleotide was tested for RNA attachment at its 5' end. It was subjected to strong alkali treatment (2 M NaOH for 16 h at 37°C), and then digested with spleen phosphodiesterase, with and without alkaline phosphatase. If the deoxyoligonucleotide had RNA on its 5' end, the alkali would have hydrolysed the RNA, leaving a deoxyoligonucleotide with a 5'-OH end. Then it would have become susceptible to spleen phosphodiesterase. Table 2, however, shows that the deoxyoligonucleotide remains resistant to spleen phosphodiesterase, even after alkali treatment, unless it is also treated with alkaline phosphatase. Therefore, the deoxyoligonucleotide does not have even a single ribonucleotide on its 5' end. If RNA was originally present on the 5' end, then the RNA must have been degraded by nucleases either before or during extraction of the deoxyoligonucleotide from the cell.

To estimate the size of the deoxyoligonucleotide, it was subjected to Sephadex gel filtration. Figure 4 shows that the deoxyoligonucleotide is eluted from a G-100 column at about

the same position as a tRNA marker. Based on these data,

TABLE 2 Effect of alkali on the 5' end of the deoxyoligonucleotide

Enzyme	% degraded to TCA-soluble	
	Control	Alkali predigestion*
Spleen phosphodiesterase	13%	23%
BAP + spleen phosphodiesterase	69%	63%

BAP, bacterial alkaline phosphatase.

*Alkali predigestion: 2 N NaOH at 37°C for 16 h. For other experimental details, see Table 1.

as well as on the fact that it is TCA-insoluble, we conclude that the chain length of the deoxyoligonucleotide is in the range of 50-100 nucleotides.

Figure 3 shows that the deoxyoligonucleotide is labelled to maximum radioactivity within 8 s. Since we know the specific activity of the ³H-T in the growth medium, and can assume steady state labelling, the number of deoxyoligonucleotides per cell can therefore be computed. We calculate that there are ten to twenty deoxyoligonucleotides per cell.

Is the deoxyoligonucleotide involved in DNA replication? The following evidence suggests that it is. First, two specific inhibitors of DNA replication, 6-(*p*-hydroxyphenylazo)-uracil (HPUra) and nalidixic acid, inhibit synthesis of the oligonucleotide. The experiment with HPUra is particularly significant because its mode of action is understood; the reduced form of the drug inhibits *B. subtilis* DNA polymerase III, an enzyme required for DNA replication^{12,13}.

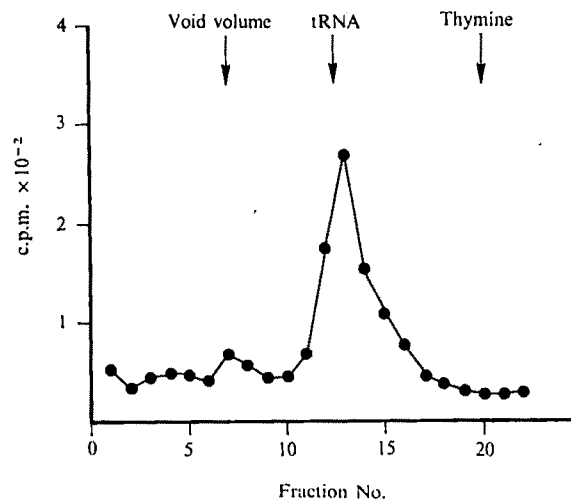


FIG. 4. Sephadex G-100 gel filtration of the deoxyoligonucleotide. Fractions containing the ³H-deoxyoligonucleotide were pooled from sucrose gradients similar to those shown in Fig. 3. They were neutralised and sedimented in CsCl to purify partially the deoxyoligonucleotide. Conditions of centrifugation in a Beckman L2-65B ultracentrifuge were as follows. The initial density of the CsCl was 1.7 g cm⁻³, SW65 rotor, 35,000 r.p.m. for 48 h at 10° C. The top 0.5 ml from each centrifuge tube was discarded and the remainder concentrated by dialysis against 30% polyethylene glycol. Finally, the deoxyoligonucleotide solution was dialysed against 0.1 M NaCl, 1mM Tris, 1mM EDTA, pH 7.0, and 0.2 ml applied to a Sephadex G-100 column equilibrated with the same solvent as above. The column size was 0.8 cm x 35 cm. Elution of the column with the solvent described above was at 25° C at 1 ml per 10 min. The void volume was determined using calf thymus DNA. The positions of wheat germ tRNA and thymine were determined by ultraviolet absorbance at 260 nm, and the position of the deoxyoligonucleotide by counting radioactivity as described in Fig. 1.

Pulse-chase experiments show that the deoxyoligonucleotide can be chased into larger DNA, although it is chased much more slowly (about 5 min) than it is labelled (Fig. 3). Because of the large deoxyribonucleotide pools in *B. subtilis* it is difficult to be sure that the oligonucleotide is chased directly into larger DNA. It could be broken down and the nucleotides re-used during the chase. There is an argument against breakdown and re-use; if cells are labelled with a short pulse of $^3\text{H-T}$ (which labels the deoxyoligonucleotide) followed by 5 min of incubation with 400 μM HPUra; the deoxyoligonucleotide is still found at its usual position near the top of a sucrose gradient. It is neither degraded nor chased into larger DNA. This suggests that the deoxyoligonucleotide is chased directly into larger DNA and not simply degraded and re-used during pulse chase experiments.

Hybridisation experiments show that the deoxyoligonucleotide hybridises with high efficiency, and equally well, to each of the separated strands of *B. subtilis* DNA; 11.3% of the radioactive deoxyoligonucleotide hybridises to the H (heavy) strand and 11.8% to the L (light) strand. (The separated strands were a gift of R. Rudner¹⁴.)

We do not understand why the deoxyoligonucleotide accumulates only after $^3\text{H-T}$ pulses (steady state labelling⁴) and not after $^3\text{H-TdR}$ pulses (non-steady state labelling⁴) in *B. subtilis*. Also, it is not observed after $^3\text{H-T}$ pulse-labelling of *E. coli* (our unpublished data and ref. 9). However, Jacobson and Lark, using T4 polynucleotide kinase to label the ends and hence count the number of chains in exponentially growing *E. coli*, found a large number of small DNA chains comparable in size to the deoxyoligonucleotide we observe⁷.

In summary, the following facts suggest that the deoxyoligonucleotide is involved in DNA replication. (1) It is labelled rapidly during pulses with $^3\text{H-T}$; (2) it can be chased into larger DNA, and (3) two inhibitors of DNA replication inhibit its synthesis. On the other hand, this evidence is also consistent with the possibility that the deoxyoligonucleotide is involved in DNA repair processes away from the replication fork. Further work is needed to determine the exact role of the deoxyoligonucleotide in DNA metabolism.

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Protease inhibitors do not block transformed cells in the G₁ phase of the cell cycle

PROTEOLYTIC treatment releases normal fibroblasts from density-dependent inhibition of growth (DDI)^{1,2} and induces surface properties characteristic of transformed cells. Furthermore, increased³⁻⁵ and altered⁶ protease activities are found in transformed cells. Also, a number of commercially available protease inhibitors including N- α -tosyl-L-lysyl-chloromethane (TLCK) selectively inhibit the growth of transformed cells⁷. All this has led to the suggestion that a protease-like activity is required by transformed cells for unrestrained growth⁷ and, by inference, that inhibition of this activity should restore 'normal' growth control (that is, DDI). In fact, TLCK lowers the saturation density⁷, de-

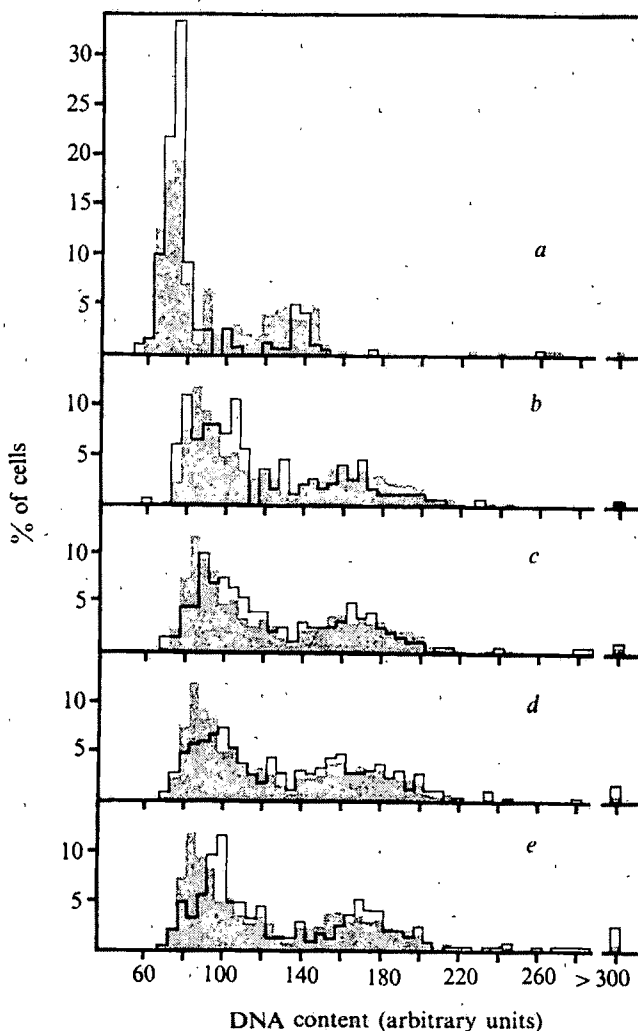


FIG. 1 DNA content of individual cells. a: 3T3 cells; shaded columns, log phase; open columns, at confluency. b-e: SV 3T3 cells; shaded columns, untreated controls; open columns, SV3T3 treated for 48 h with TLCK; b, 25 $\mu\text{g ml}^{-1}$; c, 50 $\mu\text{g ml}^{-1}$; d, 75 $\mu\text{g ml}^{-1}$; e, 100 $\mu\text{g ml}^{-1}$. All measurements calibrated with golden hamster lymphnode lymphocytes as reference (40 arbitrary units per cell).

TABLE 1 Effect of TLCK on proliferation and thymidine incorporation

Cell line	TLCK ($\mu\text{g m}^{-2}$)	Proliferation (doublings/24 h)		Mitosis per 10^3 cells	Thymidine incorporation	
		attached cells	total cells		c.p.m./ 10^3 cells (1 h pulse)	% labelled cells (4 h labelled)
SV 3T3	0	1.18 ± 0.20	1.19 ± 0.18	18.4 ± 3.8	125 ± 24	42 ± 4
	30	0.64 ± 0.23	0.66 ± 0.23	12.0 ± 2.5	119 ± 26	44 ± 5
	60	0.08 ± 0.07	0.17 ± 0.12	1.5 ± 1.4	56 ± 21	40 ± 2
	90	-0.21 ± 0.1	0.03 ± 0.03	0.1 ± 0.2	30 ± 20	42 ± 3
3T3 log phase	—	0.89	ND	12.6	105	32 ± 5
stationary phase	—	0.01	ND	0	1	2 ± 2

SV 3T3 cells were seeded in 3.5 cm Petri-dishes and allowed to grow to a density of approximately $10,000 \text{ cells cm}^{-2}$ (subconfluent). The medium was replaced and $20 \mu\text{l}$ of freshly made solutions of TLCK were added to each plate to give the final concentrations indicated. Thymidine incorporation was determined 24 h after the addition of TLCK, the mitotic and the labelling indices were determined 24 and 48 h after addition of TLCK. The number of cell doublings per 24 h was extrapolated from growth curves (5 points within 48 h). In addition to cells attached to the culture vessel, cells floating in the medium were also counted to give the total number of cells per plate. For comparison, untreated 3T3 cells in the log phase (about $20,000 \text{ cells cm}^{-2}$) and after reaching stationary phase were included in this experiment (five experiments with SV3T3; two experiments with 3T3). Figures in parentheses are % of the controls. ND, Not done.

creases the agglutinability with lectins and induces 'flat' morphology in transformed cells⁸. Here we present evidence that TLCK-inhibited cells are, however, not arrested in the G_1 phase of the cell cycle; thus, they do not meet a primary criterion⁹ for density-dependent inhibition of growth.

Freshly made solutions of TLCK (Calbiochem) were used for experiments. 3T3 mouse fibroblasts and their SV40 transformed derivative line (SV3T3) were used as the experimental cell lines. The procedures for the culture of the cells⁷, the growth inhibition studies⁷ and the thymidine incorporation⁸ have been published previously. Autoradiographs of cells grown on coverslips, labelled with ^3H -thymidine ($0.5 \mu\text{Ci ml}^{-1}$ medium; 5 Ci mmol^{-1}) for 4 h, were prepared with Kodak Bulk Emulsion NTB 2 and developed after 7 d exposure. To determine the mitotic index, cells were swollen and Orcein-stained according to the method of Fogh¹⁰. The DNA content of individual Feulgen stained cells was determined with an integrating microdensitometer (Barr and Stroud, Glasgow, Scotland). Two hundred to five hundred cells were measured per specimen at 560 nm, and the results expressed in arbitrary units. Lymph node lymphocytes from golden hamsters were placed on each of the microscope slides as reference cells.

The proliferation of SV3T3 cells is inhibited in a dose-dependent fashion by TLCK⁷; the inhibitory effect is mainly on cell proliferation and not simply due to detachment of cells from the plate (compare 'attached' and 'total' cells in Table 1), although there were some cells floating off into the medium, especially at high concentrations of TLCK. That TLCK inhibits proliferation is further supported by the fact that the reduced growth rate was paralleled by a decrease in the mitotic index (Table 1). The inhibition of cell proliferation was not, however, paralleled by a comparable decrease in DNA synthesis: even in TLCK-treated cultures where little or no net increase in cell number occurred, there was still considerable thymidine incorporation. Furthermore, the labelling index (4 h thymidine pulse) was not reduced by TLCK, although at higher concentrations of TLCK there appeared to be fewer grains (decreased by about 50%) over most of the labelled nuclei. By comparison, thymidine incorporation into 3T3 cells was drastically reduced after they became stationary (Table 1, bottom). Therefore, we conclude that TLCK does not stop transformed cells from entering the S phase.

This conclusion is further supported by measurements of the DNA content of individual cells in TLCK-treated cultures. In Fig. 1a, 3T3 cells are shown for comparison: there was a shift from the G_2 to the G_1 phase in 3T3 cells when they reached confluency. In contrast, in SV3T3 cells treated with increasing doses of TLCK (Fig. 1b-e), a shift to the G_1

phase did not occur. Rather, a dose-dependent shift to higher DNA content in the treated cells was seen. This seems to agree with the data in Table 1: the mitotic activity is reduced, but most cells continued to synthesise DNA (labelling index), although at a reduced rate.

In contrast to previous suggestions⁷, these results indicate that TLCK fails to induce density-dependent inhibition of growth in the sense that SV3T3 cells in the presence of this inhibitor are not arrested in the G_1 phase of the cell cycle.

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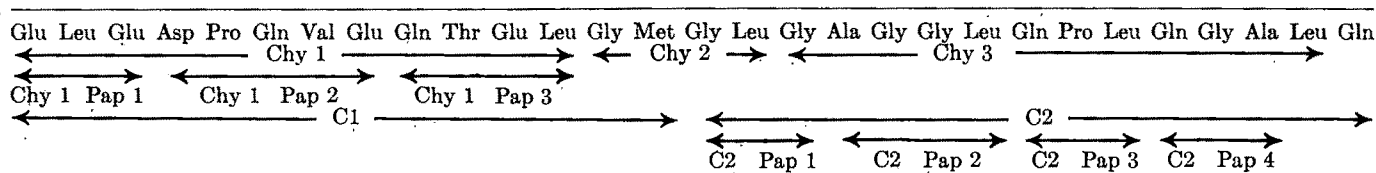
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The amino acid sequence of guinea pig C-peptide

THE discovery that the C-peptide of ox proinsulin is present in substantial amounts in the pancreas¹ has led the way to the study of C-peptides from a variety of mammalian species. The isolation and elucidation of primary structure has been described for the C-peptides of pig^{2,3}, man⁴⁻⁶, ox^{1,2,7,8}, dog, sheep and monkey⁹, rat¹⁰⁻¹², horse¹⁰ and duck¹³.

FIG. 1 Assignment of the primary structure of guinea pig C-peptide.



The horizontal arrows indicate the peptides liberated by the action of chymotrypsin (Chy) or cyanogen bromide (C) on the intact C-peptide and by the action of papain on the resulting fragments. The half arrows indicate amino acids released by stepwise degradation with carboxypeptidase A.

FIG. 2 Comparison of the amino acid sequence of the C-peptides of guinea pig and rat.

Residue number																																
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31		
Guinea pig																																
Glu	Leu	Glu	Asp	Pro	Gln	Val	Glu	Gln	Thr	Glu	Leu	Gly	Met	Gln	Leu	Gly	Ala	Gly	Gly	Leu	Gln	Pro	Leu				Gln	Gly	Ala	Leu	Gln	
Rat I																																
Val			Pro			Leu			Gly			Pro			Glu	Asp			Thr			Ala			Leu	Glu			Val	Arg		
Rat II																																
Val			Ala			Leu			Gly			Pro			Gly	Asp			Thr			Ala			Leu	Glu			Val	Arg		

The primary structure of guinea pig C-peptide is that presented in this communication; the rat sequences have been reported^{10,12}. The horizontal line indicates residue common to the three structures. The differences between the three sequences appear to involve single base changes except at residues 8, 10 and 14 in the guinea pig. Residue 14 in the guinea pig is methionine whereas glycine occupies this position in the C-peptides so far reported.

The rodents, guinea pig, chinchilla and coypu, are classified in the suborder Hystricomorpha, principally on the basis of some anatomical and physiological characteristics. A knowledge of the primary structure of their proinsulins could contribute a molecular parameter to this classification. The amino acid sequence of their insulins is already known; the guinea pig sequence¹⁴ differs in 17 positions from that of coypu¹⁵ and coypu differs in 20 positions from chinchilla (N. R. Lazarus, R. Neville, and D. G. S., unpublished data). Elucidation of the primary structure of the corresponding C-peptides may provide further insight into the phylogeny of these species. Here, we present the amino acid sequence of guinea pig C-peptide.

C-Peptide was isolated from 2 kg (wet weight) of pancreatic tissue obtained from 350 Hartley strain guinea pigs. The method employed was essentially the same as that described previously⁴. The procedure yielded 8 mg of C-peptide and the homogeneity was assessed by analytical electrophoresis², NH₂-terminal analysis¹⁶ and amino acid analysis¹⁷. The sequence of amino acids was determined by quantitative techniques involving the isolation of fragments liberated by chymotrypsin, cyanogen bromide and papain (Fig. 1) and stepwise degradation of the individual peptides. Amides were assigned by comparison of the electrophoretic mobilities of peptides⁵ and by isolation of pyrrolidone peptides derived from NH₂-terminal glutaminyl peptides.

The results allow a comparison to be made between guinea pig C-peptide and the only available rodent C-peptides, rat I and rat II (Fig. 2). The guinea pig sequence differs in 13 positions from rat I and 12 positions from rat II. Within the order Rodentia, the suborders Hystricomorpha and Rattus can be distinguished not only by their insulins but also by their C-peptides. It remains to be seen whether the structures of the hystricomorph C-peptides, when these are available, will support the existing hystricomorph classification.

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Second naturally occurring β -galactosidase in *E. coli*

SEVERAL studies have shown that the intestinal mucosa of various mammals contains at least two β -galactosidases¹⁻⁵. One of these has a primary specificity for lactose; the other has a primary specificity for synthetic β -galactosides such as o-Nitrophenyl β -D-galactopyranoside (ONPG). Since strains of *Escherichia coli* K12 bearing deletions of the *lac Z* gene have a very small residual activity toward ONPG, there must also be a second β -galactosidase in *E. coli*. We report here that *E. coli* K12 does indeed possess a second β -galactosidase. Although this enzyme has virtually no activity toward lactose, its synthesis is induced by lactose.

Strain DS4680A is a *lac* i^+ z^{del} y^+ strain. It is accordingly unable to ferment lactose and will not grow on lactose as a sole carbon source. Table 1 shows the amount of β -galactosidase activity in crude extracts of DS4680A grown in proline + IPTG (a gratuitous inducer of the *lac* operon) and grown in proline + IPTG + lactose. (IPTG is isopropyl 1-thio- β -D-galactopyranoside.) The β -galactosidase activity is induced to increase 230-fold by lactose. Since DS4680A had undergone no selection for β -galactosidase activity, this observation suggested that the activity occurs generally in *E. coli* K12 strains. We accordingly tested two additional *lac* Z deletion strains for the presence and inducibility of this activity. The tested strains (see Table 1) are unrelated to either DS4680A or to each other. In each there is a β -galactosidase activity induced by lactose (but not by IPTG), supporting the hypothesis that the gene for a second β -galactosidase does occur normally in *E. coli*.

TABLE 1 Specific activity of β -galactosidase in crude extracts of *lac* Z deletion strains

Strain	Uninduced	Induced	No. of experiments
DS4680A	0.131	30.2	3
W4680	0.112	20.9	1
2320M15	0.023	13.0	1
1011	<0.0015	<0.0015	1

Cultures were grown in phosphate minimal medium containing 0.1% proline as a carbon source, vitamin B₁, 2×10^{-4} M IPTG (uninduced) or with the addition of 0.2% lactose (induced). The cells were collected and ground with alumina and the nucleic acids precipitated with streptomycin sulphate. β -galactosidase activity was assayed at 37°C in 5 mM ONPG in 0.125 M KPO₄ buffer, pH 7.5, containing 5 mM Mg²⁺. One unit of activity is defined as the hydrolysis of 1 nmol of ONPG per min. Protein concentrations were determined by the method of Lowry *et al.*⁸ Tabulated values are in U mg⁻¹ protein. All strains are *E. coli* K12. All bear deletions within the *lac* Z gene and have functional *lac* permeases (*lac* Y^+). For purposes of comparison with the above values we have measured the specific activities of extracts of the wild type progenitor of DS4680A under these same conditions. The specific activity of an uninduced culture was 8.02 U mg⁻¹ protein; the specific activity of a culture in which the Z gene product was induced with IPTG was 11,865 U mg⁻¹ protein.

β -galactosidase II must have virtually no lactase activity *in vivo* since cultures in which the enzyme has been fully induced are not capable of growth when resuspended in lactose + IPTG minimal medium. The cells do have ample activity as measured by the hydrolysis of ONPG (10–30 U mg⁻¹) because strains can be selected that grow on lactose with as little as 16 U mg⁻¹. The enzyme is not cryptic *in vivo* because whole cells in which the β -galactosidase II activity has been induced are capable of hydrolysing ONPG. Therefore the failure of the cells to grow is probably due to an insufficient lactase activity.

β -galactosidase II has a small detectable amount of lactase activity *in vitro*. An extract of DS4680A in which the β -galactosidase II activity had been induced was prepared as in the legend of Table 1. The extract was permitted to hydrolyse lactose at a concentration of 139 mM under conditions identical to those described in Table 1. The reaction was terminated by heating for 10 min at 90°C. The amount of free galactose was assayed by the Galactostat assay system (Worthington), which uses galactose oxidase coupled with a chromogen. A unit of lactase activity is defined as the release of 1 nmol of galactose per min at 37°C. The specific activity of the extract toward ONPG was 41 U mg⁻¹ protein and toward lactose was 0.37 mg⁻¹ protein. This small amount of lactase activity detected *in vitro* is clearly not sufficient to permit the growth of cells in lactose.

By a procedure similar to that of Campbell *et al.*⁶ we have obtained derivatives of strain DS4680A that contain an evolved β -galactosidase (*ebg*⁺) which permits growth on

lactose as a sole carbon source⁷. A *lac*⁻ revertant of one of these strains was found to have lost both the ability to grow on lactose and the second β -galactosidase activity (see strain 1011 in Table 1), although it retains a functional *lac* permease. The simultaneous loss of both lactose fermentation and β -galactosidase II activity could possibly be the result of a double mutation. We have shown that *ebg*⁺ is closely linked to the marker *tolC* at 59 minutes on the *E. coli* map, there being 18.7% recombination between *tolC* and *ebg*. We crossed strain 1011 to the F⁻ *lac* Z^{del} Y^+ str^r *tolC* strain DLH-11 and selected str^r *tolC*⁺ recombinants. Sixty-eight such recombinants were scored for the presence of the inducible β -galactosidase II. Thirteen of the sixty-eight had such activity, giving a recombination frequency of 19.1% between *tolC* and the gene for the second β -galactosidase. Therefore the genetic position of the gene for the second β -galactosidase is the same as that of *ebg*⁺.

The function of the second β -galactosidase in both *E. coli* and in mammals is unknown¹, though there is the interesting possibility that their functions may be analogous.

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Detection by DNA polymerase I of breaks produced in rat liver chromatin *in vivo* by alkylating agents

In previous papers it has been shown that the *in vitro* template activity of DNA¹ and chromatin² with DNA polymerase I of *Escherichia coli* is altered by the action of ionising radiation. These changes have been attributed¹ to the formation of new binding sites on the DNA for the enzyme, some sites being active in the synthesis of DNA and others inactive. These new sites are associated with single-strand breaks in the DNA. By working under conditions either of enzyme excess or of excess of template it is possible to distinguish between newly formed active and inactive enzyme binding sites.

These studies^{1,2}, along with the work of Kornberg *et al.*³ have shown that DNA polymerase I could be of value in the investigation of single-strand breaks in DNA under non-denaturing conditions (as opposed to the denaturing conditions used in the alkaline sucrose gradient centrifugation technique). Many agents lead ultimately to single-strand breaks in DNA. Here we wish to report the use of DNA polymerase I to investigate the *in vivo* formation of single-strand breaks in the DNA of rat liver chromatin following injection of two different types of methylating agent, namely dimethylnitro-

samine (DMN) and methyl methanesulphonate (MMS). DMN is a known hepatocarcinogen in rats, producing tumours when fed as part of the diet⁴, or when injected as a single dose to neonates⁵ or to partially hepatectomised animals⁶; MMS is not known to produce liver tumours⁷. A notable feature of the present study is that after injection of these agents single-strand breaks can be detected even after very short times and after very low doses.

Chromatin was prepared from rat liver at various times following injection of known amounts of the methylating agent. Chromatin prepared from untreated rats was used as a control. The rate of incorporation of ³H-dTTP into acid-insoluble material was used as a measure of the rate of DNA synthesis and this was taken as a measure of the template activity of the chromatin.

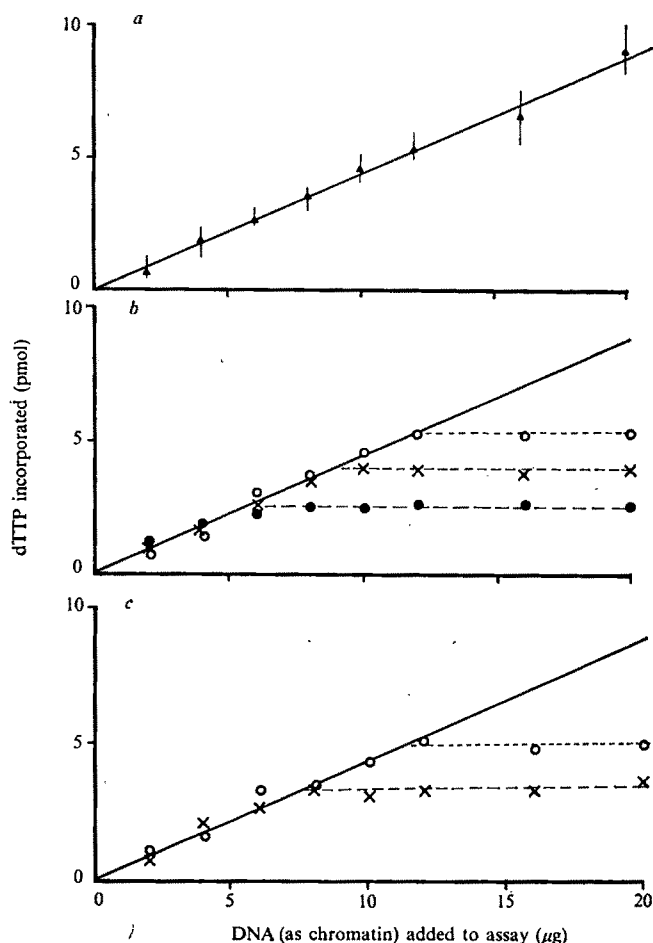


FIG. 1 Incorporation of dTTP into acid-insoluble material with increasing amounts of DNA (as chromatin) added to the assay. *a*, Control chromatin (▲); average of several independent experiments. The vertical lines indicate the range of values obtained. *b*, Chromatin following dimethylnitrosamine (DMN) treatment. The solid line shows control chromatin from *a*; ○, 2 mg kg⁻¹; ×, 14 mg kg⁻¹; ●, 30 mg kg⁻¹. *c*, Chromatin following methylmethanesulphonate (MMS) treatment. The solid line shows control chromatin from *a*; ○, 50 mg kg⁻¹; ×, 200 mg kg⁻¹. Rat liver chromatin was prepared from liver nuclei by the procedure described²⁰ for thymus chromatin: the nuclei were prepared by a modification of the method of Widnell and Tata²¹. Methylating agents were dissolved in normal saline and injected intraperitoneally. *E. coli* DNA polymerase I was prepared by a slight modification of Jovin *et al.*²²: the enzyme used was the phosphocellulose fraction that had been further purified by ultra-centrifugation in a glycerol gradient and contained no endonuclease I nor exonuclease III: 0.1 units were added to each assay. Details of the assay have been given elsewhere². Varying amounts of chromatin and water (to give a final volume of 0.3 ml) were added and thoroughly mixed before incubation at 37° C for 30 min.

The template activity of control chromatin increased linearly with the amount of DNA (as chromatin) added to the assay system up to at least 20 μg DNA per assay (see Fig. 1*a*). Under these conditions there is always an excess of enzyme over binding sites on the DNA present in the assay. Two factors preclude measurements of template activity at higher chromatin concentrations: (1) the difficulty of measuring small volumes of concentrated chromatin and (2) the large amount of precipitate since chromatin is insoluble in the assay mixture².

The template activity of chromatin from treated animals in all cases rises linearly with the amount of DNA (as chromatin) added to the assay mixture up to a certain level and is indistinguishable from controls; at higher DNA concentrations the template activity remains constant (see Fig. 1*b* and *c*). This maximum value depends, for any one sample of chromatin, upon the dose of methylating agent given, the time period between injecting the animals and preparing the chromatin, and the period of storage of the chromatin after preparation and before its assay. Figure 1 shows the dependence of incorporation of dTTP into acid-insoluble material, in 30 min, on the amount of chromatin added to the assay; in this experiment the chromatin was prepared 5 h after injection and was assayed as soon as possible (about 18 h after beginning preparation). Values are given for: (a) control chromatin, (b) chromatin prepared from DMN-treated animals, and (c) chromatin prepared from MMS-treated animals. Table 1 shows the variation of maximum template activity of the treated chromatin with dose and with period of storage of the chromatin at 4°.

Preliminary experiments (R. Saffhill, unpublished results) designed to measure the amount of DNA polymerase I that will bind to control and to treated chromatin using enzyme labelled with radioactive mercury⁸ (²⁰³Hg) have shown that chromatin from treated animals can bind at least 15 times more polymerase than control chromatin. Little, if any of this can be due to binding to chromatin protein. It has been shown that control chromatin does not bind DNA polymerase I inactively² since virtually all of the enzyme, apart from that bound actively to the chromatin is available, free in solution, for binding to poly (dAT)·poly(dAT). Further, irradiation of chromatin, which, like methylation, results in the formation of single-strand breaks, damaged bases, and apurinic sites does not produce an appreciable number of inactive enzyme binding sites². Finally, the ratio of the template activity (measured under conditions of template excess) of the DNA prepared from treated and control chromatin by centrifugation² in the presence of 2 M NaCl and *p*-aminosalicylic acid is similar to that obtained for chromatin itself, indicating that most or all of the inactive enzyme binding sites must lie in the DNA. Thus, *in vivo* treatment with methylating agents does in fact lead ultimately to the formation of single-strand breaks in the DNA that will bind DNA polymerase.

The single-strand breaks that are revealed in chromatin by DNA polymerase I are necessarily those in the regions of the DNA of the chromatin that are accessible to the enzyme. It has been shown² that only a small percentage of the DNA of chromatin is accessible to DNA polymerase and that this DNA lies within zones that are accessible to poly-L-lysine. Single-strand breaks are formed in other regions of the DNA but these would not be detectable in the present experiments. Thus, 'single-strand breaks' refer only to breaks in the regions of DNA in chromatin that are accessible to DNA polymerase I.

In vivo treatment with DMN and MMS leads (among other effects) to methylation of the chromatin: both the protein⁹ and the DNA¹⁰ are methylated. Very little is known about the methylation of the chromatin proteins apart from the fact that all of the histone fractions are methylated and the degree of methylation is considerably less than in the

TABLE 1 Effect of DMN and MMS on the template activity of rat liver chromatin

a, DMN							
Day of assay* (following preparation of chromatin)	2 mg kg ⁻¹		14 mg kg ⁻¹		30 mg kg ⁻¹		
	dTTP (pmol)†	Rel. no. of SSB‡	dTTP (pmol)†	Rel. no. of SSB‡	dTTP (pmol)†	Rel. no. of SSB‡	
Day 1	5.2	7.1	3.9	9.5	2.3	16.1	
Day 2	5.0	7.4	3.5	10.5	2.2	16.7	
Day 3	4.7	7.9	3.3	11.2	2.1	17.5	
b, MMS							
Day of assay* (following preparation of chromatin)	50 mg kg ⁻¹		200 mg kg ⁻¹				
	dTTP (pmol)†	Rel. No. of SSB‡	dTTP (pmol)†	Rel. No. of SSB‡			
Day 1	4.9	7.6	3.6	10.3			
Day 2	4.7	7.9	3.2	11.6			

* The chromatin was stored at 4°C.

† pmol dTTP incorporated in a 30 min incubation.

‡ The number of single-strand breaks (SSB) in control chromatin is taken as unity.

DNA (H. K. Cooper, thesis submitted). The sites of methylation in the DNA are the phosphodiester backbone¹¹ (producing very stable phosphotriesters¹² which are believed to be associated with the so-called X₁-products¹³ and the DNA bases¹⁴. The major methylated bases detected are 7-methylguanine, 3-methyladenine and 6-O-methylguanine, the latter being found only after treatment *in vivo* with DMN and not after MMS treatment¹³. The maximum 7-methylguanine content of the DNA is found 5 h after DMN treatment and 2-4 h after MMS treatment: at these times similar levels of 7-methylguanine are produced after injection of 2 mg DMN per kg and 50 mg MMS per kg. The methylated bases are lost from the DNA both *in vivo* and *in vitro* and their half-lives have been measured and found to be 3 d, 2-3 h (G. P. Margison, and P. J. O'Connor, personal communication) and 13 h respectively for the *in vivo* loss of 7-methylguanine, 3-methyladenine and 6-O-methylguanine. There is some evidence for the involvement of enzymes *in vivo*¹⁵ although this is presumably accompanied by some chemical depurination to yield apurinic sites in the case of the unstable alkylpurines. The nature of the excision and repair process is uncertain. It almost certainly involves the formation of a single-strand break at or near to a methylated base (or an apurinic site formed on loss of a base) at some stage followed by repair, and rejoining of the DNA. Thus, in the experiments reported here we are observing the single-strand breaks produced as part of such a repair process.

With excess enzyme, the template activity will increase linearly with the amount of DNA added to the assay, that is, with the number of active binding sites added. At lower chromatin concentrations the treated chromatin is indistinguishable from controls, which indicates that among the new enzyme binding sites produced as a result of the methylation, there are no detectable active binding sites. Inactive binding sites however, would, under these conditions of excess enzyme, merely bind an enzyme molecule that would otherwise be free in solution and not synthesising DNA, that is, there would be no detectable effect on the template activity¹.

Once the DNA concentration of treated chromatin is sufficient to provide an excess of enzyme binding sites, there is no further increase in template activity with DNA concentration. Thus the lowering of the maximum template activity (the value of which is not measurable in practice with control chromatin) arises from the formation of inactive enzyme binding sites following the action of the methylating agent.

The number of single-strand breaks in a sample of chromatin relative to the number in control chromatin can be determined from its template activity. The single-strand breaks produced *in vivo* following methylation of the DNA must all be inactive binding sites as their effect can be detected only under conditions of enzyme saturation (excess of template)¹. Under these conditions of enzyme saturation the

TABLE 2 Presence of single-strand breaks in the DNA of rat liver chromatin following *IN VIVO* treatment with DMN and MMS

Time after treatment	Relative number of single strand breaks*		
	DMN 14 mg kg ⁻¹	DMN 2mg kg ⁻¹	MMS 50 mg kg ⁻¹
Control	1.0	1.0	1.0
15 min	8.8	<4	<4
1 hour	16.1	12.6	—
2 h	17.6	9.7	7.1
5 h	9.5	7.1	7.6
12 h	—	—	10.9
24 h	7.4	5.2	14.7
72 h	4.6	—	8.4
168 h	<4	—	4.5

* The number of single-strand breaks in control chromatin is taken as unity.

template activity of a sample of chromatin is proportional to the fraction of all binding sites that are active in DNA synthesis. Although the maximum template activity for control chromatin cannot be directly measured, it can be estimated² that enzyme saturation would occur at about 80 µg DNA per assay and that the maximum template activity would be about 35 pmol dTTP incorporated in 30 min. Until sufficient single-strand breaks have been formed in the DNA to saturate the enzyme by 20 µg DNA per assay (the highest level used in the present experiments), no difference between control and treated chromatin is detectable. At 20 µg DNA per assay the condition of enzyme saturation is reached when the number of single-strand breaks has increased about 3 times. Thus, chromatin with a template activity indistinguishable from controls up to 20 µg DNA per assay is referred to here as having less than 4 times the number of single-strand breaks present in control chromatin.

The presence of single-strand breaks was investigated at different times following injection of DMN and MMS. This is shown in Table 2. The time course for two different doses of DMN (2 mg kg⁻¹ and 14 mg kg⁻¹) is similar, the number of single-strand breaks present in the DNA increasing very rapidly within the first few minutes after injection of the DMN and reaching a maximum after 1-2 h. In the case of MMS at 20 mg kg⁻¹ there is no rapid increase in the number of single-strand breaks in the DNA; instead, the increase is gradual and the maximum number is not reached until about 24 h after injection. This difference is very much greater than the error of the experiments.

The higher the dose, the more single-strand breaks are present in the chromatin 5 h after injection (see Table 1) but the increase is not linear with dose. One possible reason for this is that the degree of methylation is far in excess of the excision capacity of the cell which, presumably would be

working at maximum capacity at all doses.

The decrease in template activity (corresponding to an increase in the number of single-strand breaks in the DNA) of treated chromatin on storing could reflect some *in vitro* chemical depurination (loss of methylated purines), and subsequent hydrolysis of the apurinic sites to give inactive binding sites, certain methylated bases being known to be lost quite quickly from DNA *in vitro*¹⁶. The single-strand breaks arising from hydrolysis of apurinic sites by the proposed β -elimination¹⁰ would be expected to be inactive binding sites since they would lack the 3'-hydroxyl group that is necessary for DNA synthesis to take place³. Alternatively, the decrease could arise from the action of a contaminating exonuclease which acts upon methylated or apurinic DNA to give inactive enzyme binding sites: the enzyme being absent or not detectable in control chromatin which shows no change in template activity on storing.

In the experiments reported here we are observing the presence of single-strand breaks, within limited regions of the DNA (accessible to DNA polymerase I), which may be formed at several stages of the repair process. Other workers have followed the methyl groups remaining unexcised in the DNA^{12,15} or the single-strand breaks shown by alkaline sucrose gradient centrifugation^{17,18}, a procedure which reveals also potential single-strand breaks in the DNA (for example, apurinic sites which yield single-strand breaks under alkaline conditions).

We have shown here that within the first few minutes of DMN treatment there is a large increase in the number of single-strand breaks present in the DNA, indicating a very rapid methylation followed by a rapid removal of some of the methylated bases. The observed *in vivo* loss of 7-methylguanine cannot account for this although it is possible that the observed rates of removal of 3-methyladenine and 6-O-methylguanine could do so. Other possibilities are that other, more labile methylated bases are produced and are responsible, although these have not yet been detected (no studies have been made of the methylated base content of the DNA at very short times after injection), or that there is a very rapid enzymatic excision of some of the methylated bases from certain regions of the DNA.

The reason for the difference in the time course of single-strand breakage after DMN and MMS treatment is uncertain. It could be that the two agents methylate different regions of the DNA since they are chemically different in their mode of action: some, or all, of the regions methylated by DMN being more rapidly excised than those methylated by MMS. Another possibility is that MMS methylates and inactivates some of the enzymes involved in the repair process with a consequent delay in repair.

In summary, we have shown that methylation of DNA *in vivo* affects the template activity of chromatin for DNA polymerase I by producing single-strand breaks. A relatively large effect is detected after low doses of methylating agent and (in the case of DMN) at very short times after injection. Functional changes have not previously been found after very low doses of these agents. The results demonstrate also the usefulness of DNA polymerase I in the investigation single-strand breaks in DNA. Since the single-strand breaks produced as a result of the methylation are all inactive enzyme binding sites (they bind DNA polymerase but DNA synthesis does not take place), the 3'-hydroxyl group at the break must be blocked, presumably by a phosphate. This is indicative of the type of endonuclease acting upon the methylated and apurinic DNA. Recently endonucleases that specifically attack apurinic sites in DNA have been discovered¹⁹ but nothing is known of their specificity.

To determine the possible relevance of these results to carcinogenesis we intend to study the effect of mixture of DMN and MMS upon the template activity of chromatin prepared from various tissues, along with the effect of other

carcinogens.

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Detection and radioassay of soluble circulating immune complexes using guinea pig peritoneal exudate cells

METHODS at present available for the detection and quantitation of soluble circulating immune complexes include precipitation with Clq in agarose gel diffusion¹, precipitation with monoclonal rheumatoid factor², ultracentrifugation with inhibition of antibody-mediated lymphocyte cytotoxicity¹⁰, and precipitation with an optimal concentration of polyethylene glycol^{2,3}. These procedures either involve the use of substances isolated by fairly laborious means, or take some time to yield results. Preliminary tests in this laboratory showed that aggregated human immunoglobulin ingested by guinea pig macrophages was easily detectable by immunofluorescence, and that similar inclusions could be demonstrated after exposure of macrophages to systemic lupus erythematosus (SLE) sera.

We have used radioassay to measure uptake, and report here a new method applying the principle of competitive inhibition to the ability of guinea pig peritoneal exudate cells to ingest soluble immune complexes. The method takes only a few hours, and can detect as little as 25 ng of soluble immune complexes. Preliminary results showed the presence of complexes in twenty-two out of twenty-five randomly selected SLE sera (88%) and in none of twenty-five normal human sera. By the polyethylene glycol method, performed as described below, seventeen of the same twenty-five SLE sera

(68%) were positive for complexes as judged by the percentage of their total IgG precipitated.

Macrophages obtained about 5d after intraperitoneal injection of guinea pigs with sterile liquid paraffin were thoroughly washed in medium 199 three times by gently sucking up and down with a Pasteur pipette for 3 min and centrifuging at 500g for 7 min. The cells were then treated with human gamma globulin at a final concentration of 6%, and after another wash cellular debris was removed as described by Shortman *et al.*⁸. Human gamma globulin aggregated by heating at 63°C for 15 min was labelled with ¹²⁵I using the chloramine T method⁴ to produce labelled aggregate at a concentration of about 10 mg per ml.

Sera were tested for their inhibitory effect on uptake of labelled aggregate by the macrophages as follows. Tests were set up in triplicate in Falcon tubes containing 750,000 cells in 0.5 ml of medium 199 to which were added 10 μ l of a 1:5 dilution of serum in phosphate buffered saline (PBS), pH 7.4, and 10 μ l of the labelled aggregate solution diluted to give about 5000 c.p.s. The serum and aggregate were added simultaneously. In the control test, PBS was substituted for serum, in order to determine maximal uptake of labelled aggregate in the absence of competing serum. Cell counting was standardised by using a Coulter counter. All sera tested were decplemented by incubating at 56°C

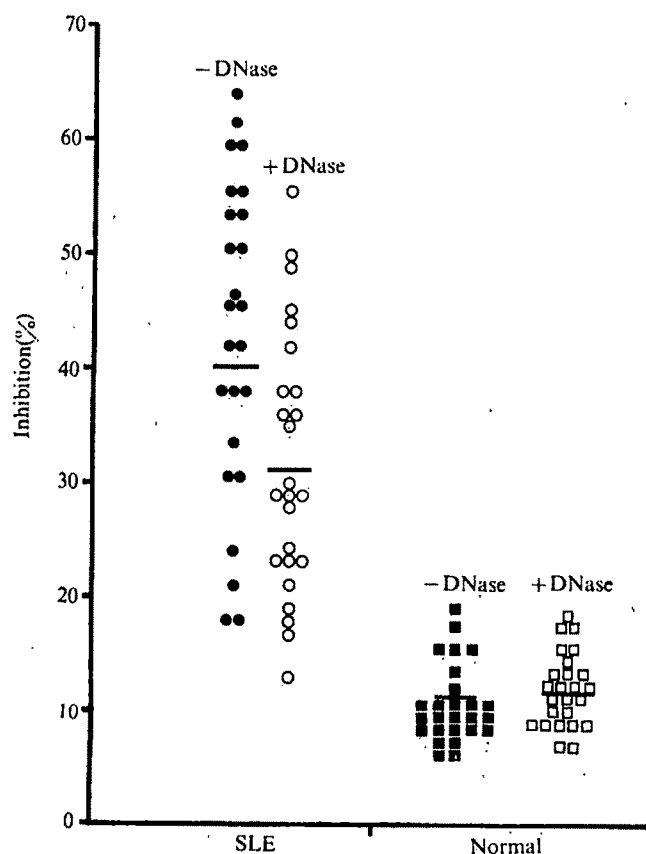


Fig. 1 Inhibition by human sera of uptake of labelled aggregated human gamma globulin by guinea pig macrophages. Percentage inhibitions and means for twenty-five SLE sera and twenty-five normal sera are shown under -DNase. Also shown is the effect of pretreatment of the sera with DNase. DNase I was added to the sera to a concentration of 50 μ g ml⁻¹, and the mixtures incubated at 37°C for 1 h in the presence of Mg²⁺. The mean inhibitory activity of the SLE sera was significantly ($P < 0.001$) reduced. As well as normal sera, sera from forty-one patients with diseases other than SLE were tested: osteoarthritis (10), ankylosing spondylitis (5), scleroderma (5), sarcoidosis (5), acute appendicitis (4), peritonitis (2), chronic bronchitis (5), carcinoma of the bronchus (2), carcinoma of the stomach (1), and carcinoma of the prostate (2). All gave inhibition within the normal range.

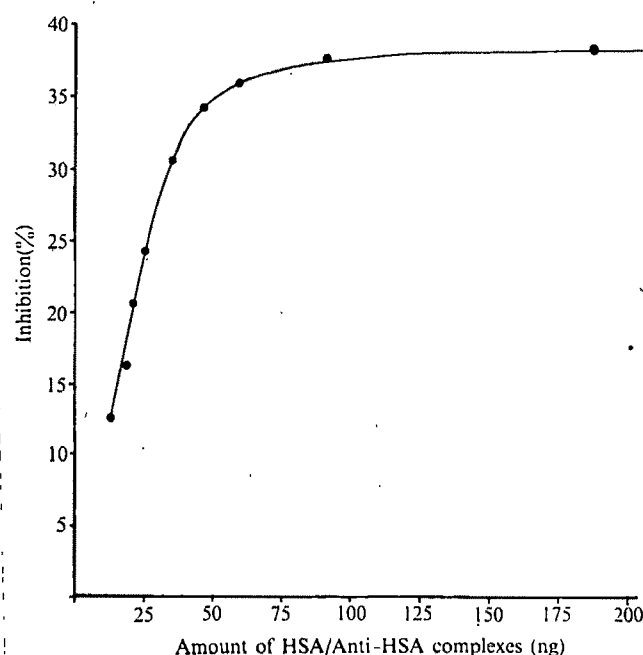


Fig. 2 Dose/response curve for inhibition of uptake of labelled aggregate by HSA/anti-HSA complexes. HSA/anti-HSA soluble complexes were prepared by determining the equivalent proportions and adding 50 times antigen excess to the antiserum. The precipitate forming after incubation was discarded and the supernatant containing the soluble complexes used in the test. The figures shown in the ordinate represent the protein content of various dilutions of the supernatant.

for 30 min, and centrifuged at 1000g for 20 min. Fresh sera, as well as sera frozen at -20°C could be used. After the addition of serum and aggregate, the tubes were incubated at 37°C for 75 min during which they were gently shaken about once every 15 min to ensure continuous and thorough mixing of the cells with the sera and aggregate. It is important to avoid mixing by inversion or the use of a rotator as this leads to loss of cells by adherence to the caps of the tubes. The cells were then washed carefully three times by centrifugation only (avoiding sucking up and down for the same reason), using medium 199 and making sure that the cells after each wash were not disturbed during the pipetting off of the supernatant. It was found best to leave a small volume of the supernatant over the pellet, and to resuspend the cells in it by a gentle shake before adding fresh washing medium. The final pellet comprising macrophages and their ingested materials was suspended in medium 199 and its radioactive content determined by counting in a Gamma Guard 150 (ICN Tracerlab).

Iodinated monomeric human gamma globulin diluted to give the same number of c.p.s. as the aggregated human gamma globulin was added to triplicate tubes treated in the same way, and the mean count was subtracted as blank.

Figure 1 shows the inhibition of uptake of labelled aggregate in the presence of competing serum, expressed as a percentage of the maximal uptake in the absence of competition. It can be seen that SLE sera exert a more pronounced inhibitory effect than normal sera. That the inhibitory effect of SLE sera is probably due to the presence of soluble immune complexes was demonstrated by an experiment in which a solution of artificially prepared complexes of human serum albumin (HSA), and rabbit anti-human serum albumin (anti-HSA), in 20% normal human serum (NHS), was substituted for SLE serum. Figure 2 shows the dose/response curve obtained by adding labelled aggregate in competition with various amounts of HSA/anti-HSA complexes in this way. The antiserum from which the complexes were prepared was added in place of PBS in the control test.

It was found that as little as 25 ng of complex exerts significant inhibition—an indication of the sensitivity of the method. With this graph as calibration, the amount of soluble complexes present in test sera is readily determined after making corrections for the dilution factor.

Polyethylene glycol (PEG) at suitable concentration precipitates soluble immune complexes. Precipitates obtained from five of the positive SLE sera by adding 0.5 ml of a solution of PEG in borate buffer, pH 8.5, to equal volumes of 1:25 dilutions of the test sera in borate buffer to reach a final concentration of PEG of 7.5% were recovered by centrifugation and redissolved in PBS. The resulting solution was inhibitory in the test whereas the supernatant serum after the addition of PEG showed no inhibition. This is additional evidence that soluble immune complexes are responsible for the inhibition.

Fractions were obtained by gel filtration of serum samples on columns of Sephadex G150, and their inhibitory activities were measured and compared with their IgG, IgM and IgA content as determined by radial immunodiffusion assay⁶. Figure 3 shows results with one SLE serum fractionated in this way; the peak of inhibitory activity occurred in fractions of larger molecular size than IgG. The fact that no IgG was detectable by the Mancini method in the earlier of these inhibitory fractions may be accounted for by the greater sensitivity of the inhibition test. It was further shown in this experiment that when the inhibitory fractions were precipitated with PEG at a final concentration of 7.5%, the resulting supernatants had lost their inhibitory activity.

In an experiment using DNase, we have shown that part of the inhibitory effect produced by SLE sera may be due to the presence of DNA/anti-DNA complexes by demonstrating a reduction in inhibition following DNase treatment. Figure 1 shows that treatment of twenty-five SLE sera with DNase reduced their mean inhibitory activity by about 25%, in agreement with the results obtained by Creighton² with the PEG method.

When complexes of HSA with ¹²⁵I-labelled rabbit anti-HSA prepared at various antigen-antibody ratios were added in competition with SLE serum in the inhibition test described above, we found (Fig. 4, shaded portion) that the relative inhibitory effect of SLE serum increased with increasing antigen excess in the competing artificial complex. As well as providing further confirmatory evidence that the inhibitory effect of this serum is due to the presence of immune complexes, this observation suggests a simple ap-

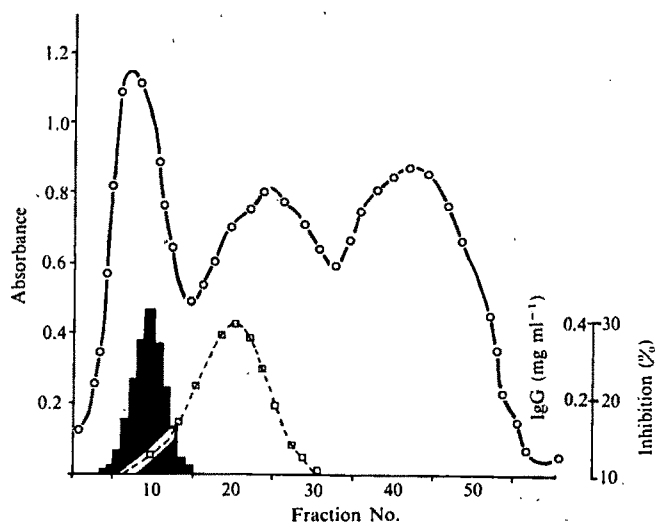


FIG. 3 Chromatography on Sephadex G150 of serum from one SLE patient showing the position of the inhibitory complexes. ○—○, Absorbance of fractions at 280 nm; □—□, IgG content of fractions; ■, inhibitory fractions.

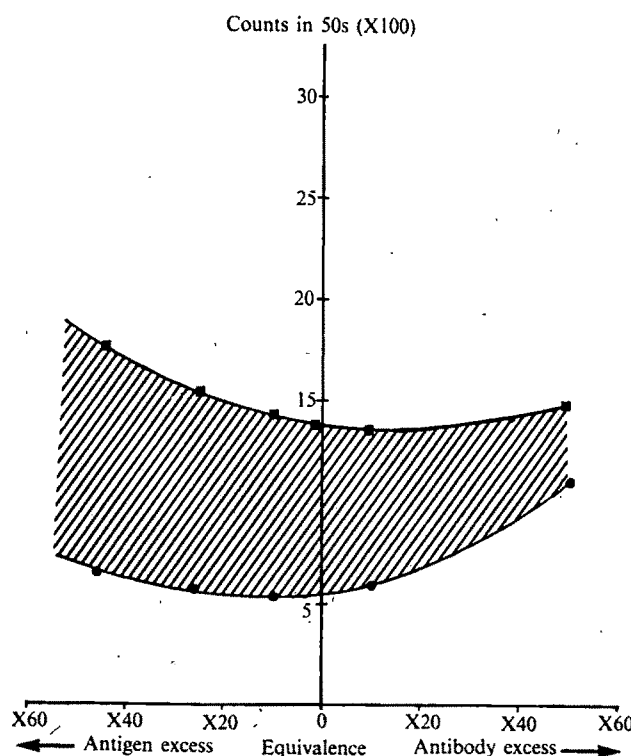


FIG. 4 The inhibitory effect of an SLE serum on the uptake of labelled HSA/anti-HSA by guinea pig macrophages. HSA/anti-HSA complexes were prepared by determining the equivalent proportions and adding 50 times, 25 times, 5 times, and 2 times antigen excess to a constant volume of antiserum. The precipitate forming after incubation was discarded and the supernatant containing the soluble complexes used in the test. ■—■, Counts in 50 s representing uptake of labelled complexes in the absence of competing serum; ●—●, counts in 50 s representing uptake of labelled complexes in the presence of SLE serum.

proach to the study of molar ratios in circulating soluble immune complexes.

Many factors may contribute to the inhibitory effect of sera on the phagocytosis of soluble labelled aggregate by guinea pig peritoneal exudate cells, including serum levels of IgG, serum complement levels, the presence of receptors for IgG and complement on the macrophage surface⁷, and the concentration of certain electrolytes, notably calcium⁸. For these reasons, the sera tested by this procedure were heat inactivated, and the cells were pretreated with human gamma globulin to 'block' surface receptors. The non-specific inhibitory effect of normal sera was thereby significantly reduced to a mean figure of 12.50% with 20% as the upper limit. The formation of soluble aggregates during heat inactivation may contribute to the inhibitory effect of sera, and very recently we have found that the use of heated sera in the test may be avoided by preliminary incubation of the cells with fresh guinea pig serum. This serves the dual purpose of 'blocking' both the complement receptors and at least some of the IgG receptors on the macrophages, and appears to obviate the need for pretreatment with foreign immunoglobulin.

The ability of foreign (guinea pig) macrophages to ingest soluble immune complexes present in human sera raises the question why human phagocytic cells do not *in vivo* by a similar process prevent such complexes persisting in the circulation, and eventually mediating tissue damage. One explanation might be that the level of circulating complexes in SLE can exceed the phagocytic capacity of blood monocytes and other phagocytes such as Kupffer cells. So far, however, we have been unable to obtain convincing evidence that human blood monocytes in SLE ingest soluble complexes *in vivo*. It is perhaps more likely that, although the

capacity of guinea pig macrophages to recognise the immunoglobulin moiety of complexes is inhibited by pretreatment with human immunoglobulin, the conditions of the test as carried out nevertheless increase the degree of denaturation of the antibody molecules in the complexes enough to enhance their ingestion.

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Evidence against recessive inheritance of susceptibility to the chronic carrier state for hepatitis B antigen

THE discovery of Australia antigen (Au), now designated hepatitis B antigen (HBAG), and its association with type B viral hepatitis stimulated application of a variety of serological assays for the detection of HBAG and anti-HBAG in pretransfusion screening of donors' blood and epidemiological work relating to transmission of hepatitis¹⁻⁶. The reasons for chronic prevalence of HBAG in apparently healthy carriers, who form an epidemiological reservoir of hepatitis B virus (HBV), remain ill-defined. Based on family studies with HBAG, Blumberg *et al* proposed a hypothesis that the persistence of HBAG for long periods is evidence of a genetic susceptibility which is inherited as a simple recessive trait^{7,8}. The extensive family data, however, included no pedigree with progeny of two parents positive for the antigen. Such a family is crucial to lend validity to the genetical hypothesis. An independent study in Italy supported the hypothesis with certain qualifications⁹.

In reviewing the mass of published family data, Petrakis made the most critical observation that only one family in Sardinia had two parents positive for HBAG and only two of their seven children were positive for HBAG; this did not support the genetic hypothesis⁹. It is tenable however that the postulated inherited susceptibility would be shown only when people are exposed to the infectious agent, and it is conceivable that the remaining five children in the Sardinian family had not been exposed to HBV. A pedigree with two HBAG carrier parents and their progeny of three children

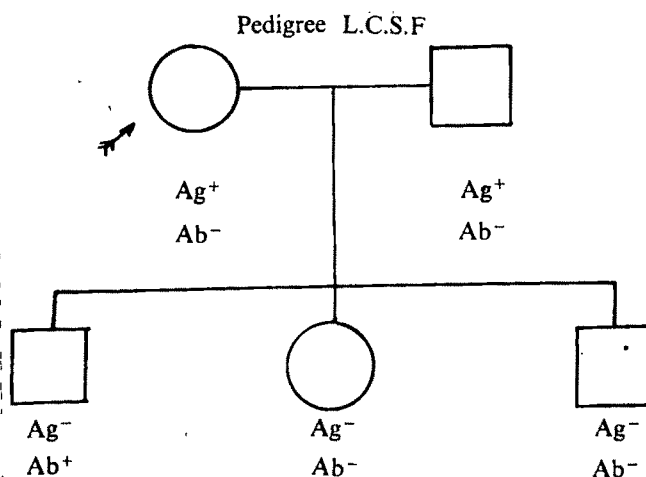


FIG. 1 A Chinese family with HBAG chronic carrier parents and a child with specific antibodies to HBAG. The other two children had neither HBAG nor anti-HBAG. Ag = HBAG; Ab = anti-HBAG.

were investigated for HBAG and anti-HBAG and the results presented in this report, provide the first discrete evidence against the genetic hypothesis.

Tests for HBAG and anti-HBAG in the sera were performed by gel diffusion analyses, counterelectrophoresis and haemagglutination assays^{4,10}. Radioimmunoassay for HBAG in the sera negative by other methods was performed according to the instructions of the manufacturers of the kits (AUSRIA™, Abbott Laboratories, Chicago). The specificity of HBAG detected in any serum was confirmed by a line of identity with a known positive control serum in gel diffusion analysis. The specificity of the antibodies was confirmed by serological neutralisation of four units of antibodies with a known HBAG-positive serum and also by 10 µg of purified HBAG¹¹. Serum samples from both parents were tested in the clinical chemistry laboratory for liver function tests.

The results of HBAG and anti-HBAG testing of each member of the pedigree are shown in Fig. 1. HBAG was consistently detected in the serum of both parents in repeated testing of their specimens obtained on three occasions over a period of six months. Both parents were in excellent health, had no apparent physical problems, and clinical or biochemical results suggested absence of liver abnormality. It was concluded that they were asymptomatic chronic carriers of HBAG¹². Both parents are immigrants from the People's Republic of China. The three children were negative for HBAG in repeated testing of their sera by every method including radioimmunoassay. The 10-yr-old son, however, had specific anti-HBAG with a titre of 256 in haemagglutination assay. The 5-yr-old daughter and 3-yr-old son had no antibodies. The absence of HBAG, but the detection of specific high titre anti-HBAG in the eldest child was considered unequivocal evidence of past exposure to HBAG. The fact that the boy failed to become a chronic carrier of HBAG but produced an immune response in the form of antibody production provides crucial evidence against the susceptibility for the chronic carrier state inherited as a simple autosomal recessive trait.

Tests for anti-HBAG in families similar to the one reported here may provide additional evidence against the genetic hypothesis. With an average incidence of three healthy HBAG-positive persons per 2,000 adults in the United States of America, the possibility of detecting such matings at random is small. The family clustering of HBAG-positive individuals is more likely a function of an increased opportunity for environmental exposure to the infectious agent than the recessively inherited susceptibility^{9,13}. These considerations may prove valuable in genetic counselling occa-

sionally sought by chronic carriers, particularly amongst informed professionals who are medically and epidemiologically more concerned about the infectivity of chronic carrier state.

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Amino acid sequence change associated with genetic marker Inv(2) of human immunoglobulin

IMMUNOGLOBULINS are proteins composed of two heavy and two light chains covalently linked by interchain disulphide bonds¹. There are two types of light chains, κ and λ . The κ type human light chain is associated with an antigenic marker called Inv (ref. 2). Three types of antigens are recognised namely Inv (1), Inv(2) and Inv(3). Ropartz, Rivat and Rousseau³ have shown that these Inv markers are inherited through a series of three alleles *Inv*^{1,2}, *Inv*¹ and *Inv*³. About 98% of Caucasoid individuals who have the Inv(1) antigen also have the Inv(2) antigen³. Since fewer than 20% Caucasoids have the Inv(1, -2) phenotype⁴ it follows that the *Inv*¹ allele is rare in this race.

The Inv markers are associated with the presence of leucine and valine in position 191. Residue 191 is leucine in the case of Inv (1, 2) and valine in the case of Inv(3) in monoclonal κ chains^{5,6} and in normal polyclonal light chains⁷. The primary sequence associated with the third antigen, Inv (1, -2), has remained unknown.

We now report studies of a κ -Bence Jones protein, Cro, which is Inv(1, -2, -3). Figure 1 shows the Inv phenotype of the kindred of the patient. It seems reasonable to assume that an *Inv*¹ allele is present in this family and that the failure to detect Inv(2) in the Bence Jones protein is not an artefact caused by the patient's disease.

Protein Cro was purified, reduced, carboxymethylated and digested with trypsin. All the tryptic peptides of the C region

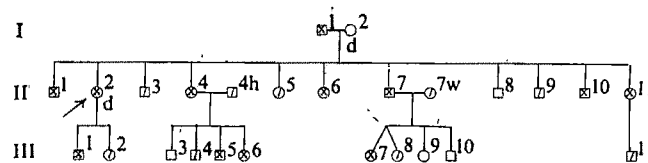


FIG. 1 Pedigree of the Cro family. X = Phenotype Inv (1, -2, 3). / = Phenotype (-1, -2, 3). Blank = not tested.

were isolated. They all had an amino acid composition identical to the corresponding homologous tryptic peptides of a κ chain except for peptide TIar. The latter peptide could be ascribed to position 150-169 by homology but did not contain alanine and it had an extra valine. The N-terminal sequence of peptide TIar was determined using the combined procedure of dansylation for N-terminal analyses and Edman degradation⁸. The result obtained was

Val-Asx-Asx-Val-Leu (Glx₅, Ser₄, Gly, Asx₂, Val, Thr) Lys

The fourth position is occupied by alanine in all the other known κ chains.

Peptide TIInBR (position 191-207) contained two leucines and two valines in its composition indicating that position 191 was leucine. In addition one of us (A.G.S.) isolated from the aminoethylated κ chain a tryptic peptide with an amino acid composition (Leu, Tyr, Ala)Cys which by homology with known chains can be situated in position 191-194.

The relation between Inv phenotype and primary structure of the C region of the κ chain is therefore as shown in Table 1. The last combination of residues has not been observed and may not exist since it seems likely that the allele Val₁₅₃Leu₁₉₁ evolved from the gene Ala₁₅₃Leu₁₉₁. Alternatively, it may correspond to the Inv(-1, -2, -3) serum reported by Steinberg *et al.*⁹

TABLE 1 Inv phenotype and κ chain structure

Inv	Residue	
	153	191
1, 2, -3	Ala	Leu
-1, -2, 3	Ala	Val
1, -2, -3	Val	Leu
?	Val	Val

All primates tested by van Loghem¹⁰ were Inv(-1, -2). Alepa and Terry¹¹ found polymorphism for both Inv(1) and Inv(3) in chimpanzees. Inv(1) has been found in baboons¹². Neither Inv(1) nor Inv(2) has been found in other monkeys. At present it is difficult to assert the evolutionary relationship between these different genes.

No allotypes in human λ chains have been described but isotypic changes have been reported in protein Kern at position 153 (ref. 13) and in the Oz locus at position 190 (ref. 14). These changes are under the control of non-allelic structural genes. The striking similarity in the location of the changes in the C region of the λ and κ chains probably means that these positions are more likely to undergo changes. Poljak *et al.*¹⁵ and Schiffer *et al.*¹⁶ have built crystallographic models of a Fab' fragment of a human immunoglobulin, New, and a Bence Jones protein respectively. In both cases it has been observed that both positions 152 and 190 occur very loosely in space. The light chain of both proteins is of the λ type but it seems likely that the tertiary structure of κ chains will be very similar if not identical to that of the λ chains. From a 2A crystallographic model (R. J. Poljak, personal communication) of the Fab' fragment of protein New it is possible to see that the positions of the λ chain, equivalent to

153 and 191 in κ chains are located on the surface of adjacent loops, exposed and at a distance of less than 10Å. Furthermore the orientation of the residues is such as to render them accessible to solvent and reagents. Our data indicate that the antiserum Inv(2) recognises leucine at position 191 encompassing position 153. The crystallographic data makes this interpretation acceptable. When position 153, alanine, is replaced by a bulkier amino acid, valine, the Inv(2) reagent is sterically hindered and cannot recognise leucine 191. Antiserum Inv(1) probably does not encompass position 153 and therefore does not distinguish the valine/alanine substitution.

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Effect of 5-fluorouracil on the gibberellic acid-induced formation of thermolabile α -amylase molecules in barley aleurone cells

In the aleurone cells of barley seed gibberellic acid (GA) stimulates *de novo* synthesis of α -amylase^{1,2}. From a study of the effect of 5-fluorouracil (FU) on the GA-evoked formation of thermolabile α -amylase molecules, Carlson³ has concluded that GA regulates a post-transcriptional control point. According to my interpretation, which differs from Carlson's conclusion, his data (Fig. 1, ref. 3) suggest that mRNAs for α -amylase are formed in the presence of GA.

Results of studies⁴⁻⁷ of nucleic acid metabolism are consistent with the concept¹ that the formation of α -amylase depends on specific RNA synthesis. Zwar and Jacobsen⁷ confirmed the finding⁵ that messenger-like RNAs are synthesised in tissue treated with GA. Other results suggest a close correlation of the synthesis of α -amylase with this effect of GA, as well as with modifications in RNA synthesis⁸ and the conversion of monosomes to polysomes⁹. Unlike actinomycin D, fluorouracil does not affect the total amount of α -amylase produced by aleurone cells incubated with GA⁷; it does not inhibit the formation of mRNAs⁷, nor does it affect the percentage of polysomes⁹ in the cells. The observed⁷ inhibition of rRNA and tRNA synthesis in aleurone cells is consistent with similar inhibitory effects of the analogue in other tissue¹⁰.

Jacobsen *et al.*¹¹ found that α -amylase produced by GA-treated aleurone cells consist of at least four isoenzymes: 3 and 4 are rendered inactive, while 1 and 2 are stable to treatment with EDTA and sodium metahephosphate. Isoenzymes 1 and 2 represent 20-25% total α -amylase activity. Calcium is an integral part of all four isoenzymes and 1 and 2 are thought to bind Ca²⁺ very strongly. Carlson³ found that when Ca²⁺ was removed from purified α -amylase (presumably from isoenzymes 3 and 4 only) the thermolability (denaturation at 72° C followed by renaturation with addition of Ca²⁺) of the enzyme formed in the presence of FU was greater than that of the 'native' (without FU) enzyme. The thermolability curves (Fig. 1, ref. 3) show that the percentage of thermolabile α -amylase molecules in the purified preparations either increased or decreased depending on whether the tissue was incubated with FU before or after the addition of GA. For example, the data (Fig. 1, ref. 3) obtained when the preparations were subjected to 72° C for 15 min show that 91% of the enzyme molecules produced by the tissue treated with FU for 72 h were thermolabile (Table 1b). Under identical conditions of denaturation 60% of the native enzyme (Table 1a) was also thermolabile. Carlson³ assumed that the thermolability of the α -amylase resulted from a misreading of the FU-containing RNA templates. Consequently, the enzyme preparations were expected to contain, in addition to normal molecules, thermolabile molecules formed on FU:RNA templates. Therefore, the change in the time (min) required to inactivate 50% of the enzyme (T50%) is also a measure of the increase or decrease in thermolabile enzyme molecules in the preparations. Table 1 shows that the T50% of the native α -amylase molecules decreased from 12 to 5 min when the enzyme molecules were formed by tissue incubated with FU continuously for 72 h. These findings raise the question of whether FU:RNA templates for synthesis of α -amylase are formed before or after addition of GA.

Since the tissue treated with FU for 72 h produced 91% labile enzymes, 83-85% of the molecules were formed on FU:RNA templates made by the tissue incubated with GA and FU together for 24 h (Table 1d and f). The data from the reciprocal experiment (Table 1c and e), in which the tissues preincubated (no GA) with 10⁻⁴ FU for 24 and

32 h respectively, produced 70 and 76% thermolabile α -amylase molecules, suggests that the FU:RNA templates were formed before the addition of GA. These tissues were preincubated in the presence of 2.5×10^{-4} uracil, which decreased the percentage of thermolabile molecules from 83 to 76% in 24 h and from 85 to 70% in 32 h. An 8-h pulse of uracil given to a tissue preincubated with FU for 24 h dilutes the pool of analogue (U:FU ratio increases from 4.7 to 77.0) and increases the FU-containing acid-insoluble RNA species⁸.

TABLE 1 Effect of 5-fluorouracil on percentage of thermolabile α -amylase molecules produced by GA-treated aleurone cells

Treatments	Thermolabile enzyme (%)	T50%
(a) Control (no FU)	60	12.0
(b) FU present for 48 h before and during the 24 h treatment with GA	91	5.0
(c) FU present for 24 h before, U present during the 24 h treatment with GA	76	5.5
(d) FU present for 24 h with GA	83	5.0
(e) FU present from 32 to 8 h before GA application, U present during 24 h treatment with GA	70	8.0
(f) FU present for 8 h before, and during 24 h treatment with GA	85	5.0

The percentage of enzyme activity that was stable on heating the EDTA-sodium metaphosphate-treated enzyme preparations at 72°C for 15 min was obtained from Fig. 1, ref. 3. The T50% value, obtained by line intercept of the thermolability curves (Fig. 1, ref. 3) represents the approximate time (min) required to inactivate 50% of the enzyme activity at 72°C. Experimental details are described in ref. 3.

Does the increase in FU-containing RNA species represent the formation of FU:RNA templates? The mRNA is a very small percentage (about 1%) of the total cellular RNA⁷. Although FU inhibits (about 50%) the synthesis of ribosomal and tRNA, it has no effect on the formation of mRNAs or on the total α -amylase produced by the GA-treated cells⁷. α -Amylase is only one of many hydrolases produced by the aleurone cells treated with GA. Time course studies have revealed an actinomycin D-sensitive phase before the formation of α -amylase^{1,12} and protease¹³. The decrease in the percentage lability (from 85 to 70%) and the increase in the T50% value (from 5 to 8 min) resulting from the addition of uracil therefore suggests that enzyme molecules similar in properties to the native enzymes (thermolability 60%, T50% 12 min) are formed in the presence of uracil and after the addition of GA. Since the tissue incubated with FU and GA together for 24 h produced 83% thermolabile molecules compared with 91% in the controls (FU for 72 h), the 'logical' conclusion is that specific FU:RNA templates for the synthesis of thermolabile α -amylase molecules are formed after addition of GA.

In the absence of statistical data (Fig. 1, ref. 3), the differences in the percentage thermolability of the different enzyme preparations (Table 1) are difficult to assess. The decrease in the time required to inactivate thermally 50% of the enzyme molecules as a result of incubation with FU and GA together (Table 1b, d and f), suggests that the FU-containing RNA templates for the synthesis of thermolabile α -amylase molecules are formed after addition of GA. The validity of such a conclusion is based on the assumption that FU:RNA templates allow for the formation of thermolabile enzymes which are otherwise identical in isoenzyme composition, Ca²⁺ binding and other properties to the native molecules. Carlson's discovery³ that FU allows the formation of thermolabile α -amylase molecules, and the finding⁸ that

alterations in nucleic acid methylation prevent enzyme formation, support the suggestion¹⁴ that in the aleurone cells the action of GA involves specific structural modifications of the transcribed substance(s) (RNAs), and consequently a translational modulation of protein synthesis.

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Intensity Fluctuation Spectroscopy of Motile Microorganisms

LIGHT microscopy indicates that in *Escherichia coli* translational motility ('running') is accompanied by considerable side-to-side or wobble motion. Moreover, in the case of chemotactic strains Berg and Brown¹ showed that a typical bacterium spends considerable time in a 'twiddling' state in which translation ceases in favour of jittering rotational motion. In both the running and twiddling state the amplitude of the non-translational component of motion is comparable with λ , the wavelength of light. It is reasonable to expect therefore that the dynamics of both wobble and twiddle motion can be studied by intensity fluctuation spectroscopy² (IFS) of scattered laser light. We have examined the effects of wobble motion on the time dependence of the fluctuating light intensity scattered by a non-chemotactic mutant of *E. coli* whose motion is dominated by running with almost no twiddling. We conclude that IFS is sensitive primarily to the rotational component of the motion.

Nossal³ has demonstrated theoretically that under certain conditions IFS is sensitive to the distribution of translational swimming speeds for motile microorganisms. These conditions are: (a) that the particles swim at a constant speed for distances not less than λ ; (b) that the amplitude of non-translational motion is small compared with λ , and (c) that the particles are small compared with λ and (or) spherically symmetric. Although Nossal *et al.*⁴ presented experimental evidence that these assumptions are met in wild type *E. coli*, Schaefer⁵ was unable to reproduce these results, attributing the discrepancies to wobble motion superimposed on translation. Nossal and Chen⁶ subsequently studied the dependence

of motility of *E. coli* on chemoattractant concentration. They assumed that reasonably accurate mean-square translational speeds can be obtained from the initial decay of the intensity correlation function of the scattered light intensity.

In this article we establish the importance of rotational motion by two lines of evidence. First, we discuss a theoretical model in which motility is modelled as a form of uniform helical motion. This is applied to IFS data obtained on a non-chemotactic mutant of *E. coli* (*cheC497*) whose motion seems to be uniformly helical under most conditions (this type of helical motion would appear as side-to-side wobble under a microscope). Although the model is too restrictive for quantitative application, it establishes that IFS is relatively insensitive to the translation swimming speed of *E. coli*. This has been confirmed under viscosity conditions such that wobble motion is suppressed.

A bacterium is considered here to be rod-shaped with length b and negligible width. The body is presumed to simultaneously spin and translate, the body fixed at an angle α with respect to the direction of translation (x axis in Fig. 1). The

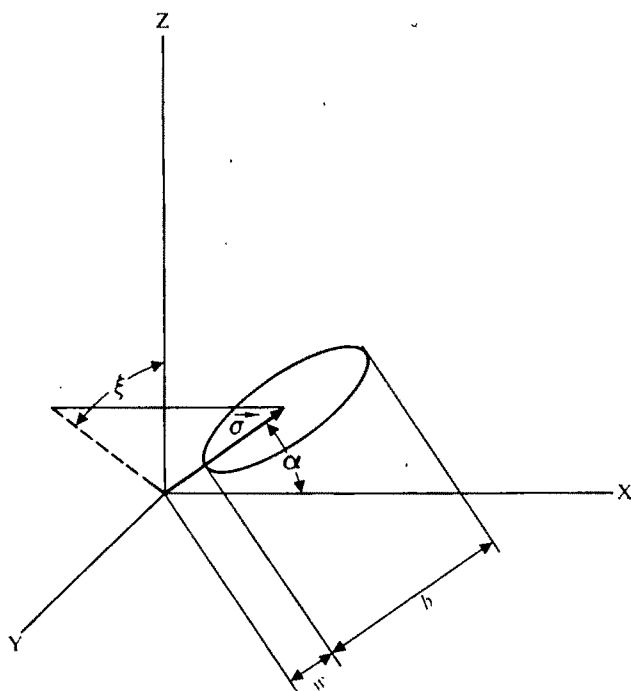


Fig. 1 Coordinate system used in model calculations.

body rotates about the translation direction with a single frequency Ω ; $\xi(t) = \Omega t$, where t is time. The intersection of the body axis (σ) and the translation axis is a distance w from the posterior (or equivalently anterior) end of the bacterium.

We make two important assumptions. First, rotational and translational motion are uncorrelated. Although this is not so for helical motion, expansion of the intensity correlation function about $t=0$ indicates that the result obtained below is similar to that expected for correlated motion. Second, to retain tractable equations it is necessary to neglect interference of light scattered from different parts of the same particle. This assumption is sufficiently restrictive that, for asymmetric bacteria, the results can only be used to demonstrate the relative importance of rotational and translational motion.

Within the framework of our model, $R_E(t)$, the correlation function of the scattered light field, takes the following form:

$$R_E(t) = \langle \exp\{i\mathbf{K} \cdot [\mathbf{R}(t) - \mathbf{R}(0)]\} \rangle \left[\frac{1}{b} \int_w^{b+w} d\sigma \langle \exp\{i\mathbf{K} \cdot [\sigma(t) - \sigma(0)]\} \rangle \right] \quad (1)$$

The corner bracket indicates an ensemble average. For incident light of wave vector of magnitude K_0 , $K = 2K_0 \sin \Theta/2$, where Θ is the scattering angle. \mathbf{R} is the position of the intersection of the body axis and the translation axis and σ is the position of the scattering element relative to \mathbf{R} .

If angular and translational motion is uniform for distances large compared with K^{-1} then equation (1) reduces to

$$R_E(t) = [1/4\pi b] \int_w^{b+w} d\sigma \int_0^{2\pi} d\phi \int_0^\pi d\theta \sin \theta \exp[2iK\sigma \sin \phi \sin \theta \sin \alpha \sin(\Omega t/2)] \times \int_0^\infty dV_x [\sin(KV_x t)/KV_x t] P(V_x) \quad (2)$$

where θ and ϕ are the polar and azimuthal angles of \mathbf{K} with respect to the x axis. $P(V_x)$ is the swimming speed distribution, V_x being the projection of the centre-of-mass velocity on the translation axis. For $t \ll \Omega^{-1}$,

$$R_E(t) = \frac{Si[K(b+w)\Omega t \sin \alpha] - Si[Kw\Omega t \sin \alpha]}{Kb\Omega t \sin \alpha} \times \frac{Si(KV_m t)}{KV_m t} \quad (3)$$

where Si is the tabulated sine integral⁷. In deriving equation (3) $P(V_x)$ has been assumed to be uniform up to a maximum speed V_m (the final results are insensitive to this distribution). Since $(b+w)\Omega \sin \alpha$ is the speed of the tip of the spinning organism, the rotation factor of equation (3) will be important whenever the tip speed is comparable with or greater than V_m .

Several observations are pertinent to equation (3). First, measured correlation functions should scale when plotted as a function of Kt if the assumptions of our model are met. Second, breakdown of Kt scaling indicates either high rotational frequencies, nonuniform motion over distances of the order of K^{-1} , or possibly particle asymmetry. Third, $R_i(K, t)$, the experimentally measured photocurrent correlation function, is proportional to the intensity correlation function rather than the field correlation function. The time-dependent part of $R_i(K, t)$, however, is proportional to $|R_E(t)|^2$ so that experimental data are compared with the square of equation (3).

Our experimental work involved measurement of the $R_i(K, t)$ for light scattered from a non-chemotactic strain of *E. coli* (*cheC497*)^{1,8}. The bacteria were studied under viscosity conditions conducive to both normal motility (showing significant non-translational motion) and motility in which the amplitude of off-axis motion was suppressed. In either case, motility in *cheC497* is dominated by running with little twiddling¹.

Samples were grown and studied in closed tubes containing Hutner's medium + 0.5% dextrose⁹. Samples were grown initially at 22° C and then at 30° C for at least three generations. All results were obtained at 30° C during the stationary phase, when the decay time of $R_i(K, t)$ showed a broad (1–3 h) minimum. We used high density populations (absorbance ~0.5) to avoid complications due to fluctuations in the total number of bacteria in the scattering volume^{5,10}. The incident wavelength was 6,328 Å and the experiments were performed with a SAICOR 43A correlator. A dilution experiment was performed as a control. A typical sample was diluted by a factor of four with air-saturated distilled water. No immediate change was observed in $R_i(K, t)$, suggesting that neither interparticle interactions nor small changes in oxygen concentration significantly affected our results.

The bacteria were also observed by light microscopy. Wobble motion with $\langle \alpha \rangle \sim 35^\circ$ was observed during all phases of growth (except in solutions containing Methocel). We tried to determine $\langle \Omega \rangle$ using stroboscopic illumination. Although high translational speeds precluded accurate measurement of $\langle \Omega \rangle$, angular velocities exceeding 10^2 rad s⁻¹ were observed for certain individuals.

Figure 2 shows $R_i(K, t)$, the measured photocurrent correlation function, at several scattering angles for samples in culture medium (viscosity = 0.82 cP at 30° C). The very short characteristic times of these curves indicate that the decay

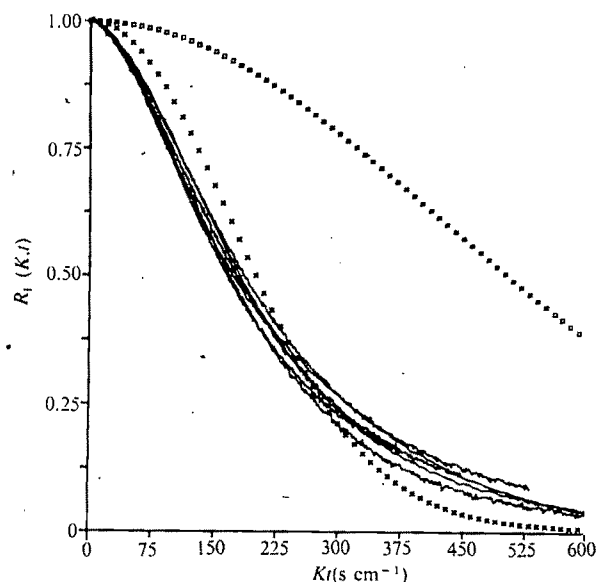


Fig. 2 Measured photocurrent correlation for light scattered from *E. coli cheC497* at $\Theta=20^\circ, 35^\circ, 50^\circ, 70^\circ, 90^\circ$ (solid lines) compared with calculated curves for pure translation (squares, $\alpha=0$, $V_m=50 \mu\text{m s}^{-1}$) and helical motion (crosses, $\alpha=35^\circ$, $b=2.7 \mu\text{m}$, $w=0.3 \mu\text{m}$, $\Omega=60 \text{ rad s}^{-1}$, $V_m=50 \mu\text{m s}^{-1}$).

was not caused by translational motion since analysis based on a pure translational model ($\alpha=0$) yields average speeds of more than $100 \mu\text{m s}^{-1}$, which are unreasonably large. Also, Kt scaling predicted by equation (3) was observed. This scaling was not observed for scattering angles larger than 90° or for other strains of *E. coli*.

Two theoretical curves are presented in Fig. 2. The squares represent the square of equation (3) assuming rectilinear motion ($\alpha=0$) and the crosses are calculated for helical motion with $b=2.7 \mu\text{m}$, $w=0.3 \mu\text{m}$, $\alpha=35^\circ$, $\Omega=60 \text{ rad s}^{-1}$ and $V_m=50 \mu\text{m s}^{-1}$. Although our theory is not expected to predict the curvature, a comparison of the characteristic times of these curves indicates the dominance of non-translational motion. The significance of Kt scaling displayed by these data cannot be established by reference to equation (3), due to the neglect of intraparticle interference.

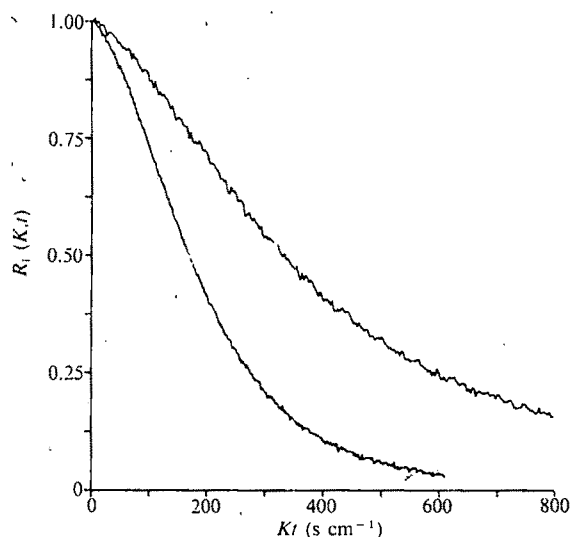


Fig. 3 Comparison of photocurrent correlation function of light scattered from *E. coli cheC497* at different solution viscosities. Data were taken at 30°C with $\Theta=70^\circ$. Upper curve, 1.6 cP; lower curve, 0.8 cP.

The most conclusive evidence for the importance of rotational motion was established by comparison of data taken from different solution viscosities. Figure 3 shows $R_1(K, t)$ for light scattered at 70° scattering angle from samples grown in the culture medium and in this medium plus 0.2% hydroxypropyl methyl cellulose (Dow Methocel 90 HG, viscosity 1.6 cP at 30°C). In the latter medium the translational speed is 20% greater, and the wobble motion is almost completely suppressed¹¹. Thus, if translation were dominating the decay of $R_1(K, t)$ a shorter characteristic time would be observed in 0.2% Methocel. In fact, a considerably longer characteristic time is found consistent with the decreased amplitude of off-axis motion ($\langle \alpha \rangle$ smaller). This establishes the dominance of non-translational motion at low viscosities. Considerable variability ($\pm 20\%$), was observed for the decay times in 0.2% Methocel, making it difficult to establish whether Kt scaling was observed.

Our results contrast with those obtained by Nossal *et al.*^{4,6} who were able to grow non-rotating cultures. We were unable successfully to repeat their experiments because (a) cultures showed great variability when collected in the log phase; (b) centrifugation led to greatly increased correlation times due to non-swimming bacteria; (c) number fluctuations¹⁰ often obscured the results at low population densities, and (d) cultures grown below 30°C had noticeable numbers of non-swimming bacteria.

We conclude that in most cases IFS is not directly sensitive to the translational speed of motile microorganisms such as *E. coli*. Although this diminishes the utility of IFS for study of centre-of-mass motion, it facilitates study of rotational motion, which is important in the understanding of flagellar action¹¹ and chemotaxis^{1,12}. Furthermore, since rotational frequencies are likely to scale with translation speed for a given solution viscosity, IFS can still be used to study the effects of environmental parameters (temperature, chemo-attractant concentration⁶) on swimming speeds.

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Is craniofacial asymmetry and adaptation for masticatory function an evolutionary process?

MANY workers have investigated asymmetry of primate skulls. Woo¹ carried out direct chordal and arcual measurement on a large number of human skulls from the 26th to the 30th Egyptian dynasties. He found the bones of the cranium exhibited an asymmetry with the right side being larger, reflecting the development of the right hemisphere of the brain. The contralateral side of the facial complex exhibited an asymmetry with the left zygoma and left maxilla being larger. The lower third of the face was not investigated.

Mulick² investigated human facial asymmetry using cephalometric skull radiographs with a three-dimensional grid system of analysis and reported a facial asymmetry in six same-sex triplets, with the larger side being to the left.

Groves and Humphrey³ reported an asymmetry of gorilla skulls (*Gorilla gorilla beringei*) with the left side exhibiting a marked increase in length from the temporal fossa to the gnathion. They postulated that such asymmetry may be consequent to an asymmetry of function of the masticatory system.

The skull complex consists of numerous constituent parts. It is, therefore, the degree of harmony between the parts which determines the symmetry of the whole. The following investigation into human facial asymmetry was devised to establish a method for analysis of overall facial asymmetry in terms of its component parts, each of which can individually vary between the right and left sides.

Sixty posterior-anterior cephalometric skull radiographs of normal children were traced. No child with a degree of clinically evident or unacceptable facial asymmetry or gross deviation of dental arrangements was included.

Six bilateral and four single roentgenographic landmarks were delineated. The single landmarks were: sella; anterior nasal spine; incisal point; menton. The bilateral landmarks were: orbitale; centre of condylar shadow; zygomatic point; upper molar point; gonion; superior extent of condyle.

To assess the relative asymmetry of the component parts of the facial complex, a method of triangulation was used. The roentgenographic landmarks were joined to form triangles on both sides of the midline, representing the right and left mandibular regions, lower, middle and upper maxillary areas and the cranial base regions. The sides of the triangles were measured to the nearest 0.5 mm. and the areas compared with the areas of the equivalent triangles of the contralateral side.

The investigation revealed an overall asymmetry in most cases with the larger side to the left. The cranial base region, lower maxillary region and mandibular region exhibit a left sided excess. The maxillary region showed a right sided excess and the dento-alveolar region the greatest degree of symmetry.

The findings are of interest in that they suggest a compensatory adaptation during growth to effect an integration of the facial components. Scott⁴ suggested that the facial skeleton should be considered as a unit built up of a number of semi-independent regions, each with its own pattern of growth and development. The orbits, nasal cavities and lower border of the mandible show a high degree of independence and are under genetic control in their determination with the dento-alveolar region and lower parts of the nasal cavities showing a greater response to functional variation. These suggestions may be supported by the present findings. The mechanism whereby this occurs may be part of an evolutionary process. Adaptation of the dento-alveolar structures to muscle pressures is well recognised.

It is reasonable to assume that optimal function is provided by maximum cuspal interdigitation of teeth. We accept that this relationship can be arrived at in occlusion (that is, with

the teeth together) even though facial asymmetry may still exist. If in the rest position, or in the habitual postural position of the mandible, the upper and lower teeth are not co-incident about the sagittal plane, then an asymmetrical functional activity of both temporomandibular joint mechanisms must compensate during chewing and non-chewing activities in which the teeth are approximated. This in clinical practice is frequently related to pain and dysfunction and is therefore not a normal adaptation in humans.

To enable bilaterally symmetrical function and maximum intercuspation of the teeth to occur, compensatory changes seem to be operating in man in the growth and development of the dento-alveolar structures which minimise the underlying asymmetry in the spatial arrangement and size of the jaws.

This factor whilst no longer being essential for man's survival with his modern diet may nevertheless be regarded as a possible factor in the evolution and natural selection processes of the subhuman species.

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Deltatheridium and Marsupials

THE evidence for the radical transfer of the famous genus *Deltatheridium* to the Marsupialia seems to be partly different from that already presented¹. The cheek tooth formula, a key character separating marsupials and placentals, is questionable for *Deltatheridium*. There are seven cheek teeth, as usual in both groups, and the fourth is molariform, as in marsupials. This is evidence on phyletic affinity only if the primitive state, that of the latest common ancestor, was otherwise. In a manuscript that has circulated privately since 1963 I have argued from diverse evidence that the seven cheek teeth of each group may well be directly homologous with those of the other, with an ambiguity as to the permanent or deciduous premolars. In other words, P₄ or DP₄ of placentals may well be homologous to M₁ of marsupials. If so, a more or less molariform state of the fourth cheek tooth is primitive to both groups and the often nonmolariform state in more or less primitive placentals is secondary. This was why I suggested² that *Deltatheridium* might have one more molar than previously thought; the suggestion is now confirmed. Relative wear of the teeth is a useful but unreliable criterion (ref. 3, footnote on page 86).

Positive evidence, however, comes from the fact that there is a sharp morphological break between the third and fourth cheek teeth. This is characteristic of marsupials but not of primitive placentals or the pantothere quasi-ancestor of both groups, *Peramus*⁴.

If *Deltatheridium* is a marsupial, as seems entirely possible, it is specially similar only to the Stagodontidae^{5,6} among possible relatives. Butler and Kielan-Jaworowska ascribe this similarity to parallelism, but there seems no positive evidence of such. Normal marsupial styler cusps are retained in *Didelphodon* but not *Deltatheridium*, so some divergence is indicated. I prefer to classify the ancestors of marsupials and placentals as marsupials, which increases the probability of allocation of *Deltatheridium* to marsupials without affecting the phyletic evidence.

It should be noted that some time ago⁷ I abandoned my ordinal name, *Deltatheridia*⁸ (I now use *Hyaenodonta*), without change in the phyletic and adaptive evidence^{3,9,10} used to establish the order. Dr Kielan-Jaworowska showed me her new material on a recent visit but my comments are based on the published data.

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Temperature-sensitive mutations affecting the regenerative sodium channel in *Drosophila melanogaster*

IN an attempt to elucidate the functions of the nervous system using behavioural mutants of *Drosophila melanogaster*, several sex-linked temperature-sensitive paralysis mutants have been isolated^{1,2}. These mutants are reversibly paralysed at 29° C while remaining normal at 22° C. The author set out to determine the molecular basis for the paralysis in one allelic series of these mutants, *shibire* temperature-sensitive (*shi*^{ts})².

A number of pharmacological agents are available which block particular functions of the nervous system. Here I report the effect of one such agent, tetrodotoxin (TTX), on the *shi*^{ts} mutants. This toxin is known to specifically block the regenerative sodium channel of the action potential mechanism³.

Varying concentrations of TTX in 0.05 M citrate buffer pH 4.8 were fed to wild-type and *shi*^{ts} flies at 22° C. After 20 h, these flies were transferred to vials containing fresh food and 12 h later the number of live and dead flies was counted. In this way, kill curves were determined for the allele *shi*^{ts} and wild-type Oregon-R flies. The 12 h delay prior to scoring allowed those flies which were initially debilitated by the treatment to either recover or die. In most cases, debilitated flies recovered during this interval and so only two classes of fly were counted, dead or alive. The kill curves obtained for these two strains are shown in Fig. 1. The *shi*^{ts} flies are far more resistant to TTX than their wild-type counterparts, the LD₅₀ of *shi*^{ts} being three times greater than that of the wild-type strain.

To show that the result observed was due to the mutation at the *shi* locus and not just the genetic background of this strain, all of the other five *shi*^{ts} alleles² were tested for their resistance to the toxin. Table 1 shows the effect of feeding TTX at a concentration of 5 µg ml⁻¹ to flies carrying each of the *shi*^{ts} alleles and including a wild-type control. As might have been expected, the alleles defined as more extreme², *shi*^{ts1} and *shi*^{ts2}, conferred the greatest resistance while the least extreme alleles, *shi*^{ts4} and *shi*^{ts5}, exhibited very little resistance. Two unexpected results were that *shi*^{ts3}, an allele with an intermediate paralytic phenotype, was more resistant to TTX than *shi*^{ts1}, and *shi*^{ts6}, which is

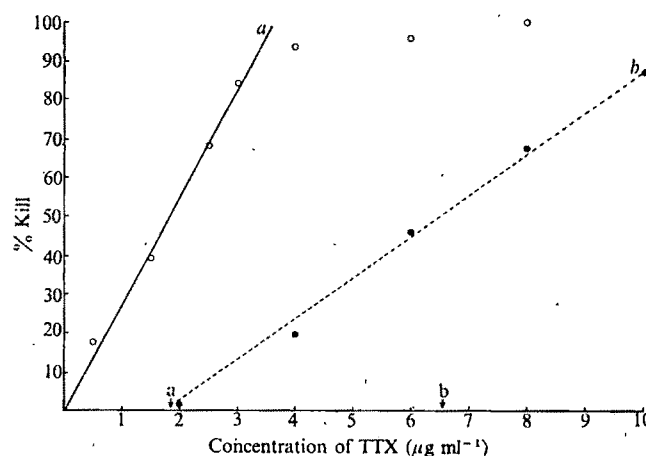


Fig. 1 Effect of increasing concentrations of TTX in 0.05 M citrate buffer pH 4.8, on a, Oregon-R, and b, *shi*^{ts} flies at 22° C. Incubation time was 20 h. Each point represents ten vials, each vial containing fifty flies. For either *shi*^{ts} or Oregon-R flies the variation in kill between vials at the same TTX concentration was always less than 5%. Arrows marked a and b indicate LD₅₀ for Oregon-R and *shi*^{ts} flies respectively.

normally considered a strong allele, was the least resistant. Other studies on the paralysis and lethality of various heterozygous combinations of the *shi*^{ts} alleles have, however, shown that in *shi*^{ts1}/*shi*^{ts1} heterozygotes, the *shi*^{ts1} phenotype is expressed while in *shi*^{ts1}/*shi*^{ts2} flies, the *shi*^{ts2} phenotype is dominant (T. C. Kaufman and D. T. Suzuki, in preparation). In fact, *shi*^{ts5} and *shi*^{ts6} are recessive to all of the other *shi*^{ts} alleles. Furthermore, *shi*^{ts1} and *shi*^{ts2} are the only alleles which are viable in heterozygotes carrying a deficiency for the *shi* locus (T. C. Kaufman and D. T. Suzuki, in preparation). Thus, the sensitivity to TTX suggests a difference in properties of *shi*^{ts1} and *shi*^{ts2} which are corroborated by other criteria.

TABLE 1 Effect of TTX on *shi*^{ts} Alleles

Genotype	No. of flies		% kill
	Dead	Alive	
Oregon-R	300	11	96.5
<i>shi</i> ^{ts1}	188	284	39.8
<i>shi</i> ^{ts2}	156	344	31.2
<i>shi</i> ^{ts3}	210	285	42.5
<i>shi</i> ^{ts4}	308	184	62.6
<i>shi</i> ^{ts5}	298	190	61.1
<i>shi</i> ^{ts6}	392	103	79.1

5 µg ml⁻¹ TTX was used on mixed male/female populations. No preferential killing of males or females was observed.

Heterozygous *shi*^{ts}/+ flies have also been tested for their TTX resistance. Table 2 shows the effect of 5 µg ml⁻¹ of TTX on these flies as compared to *shi*^{ts1}/*shi*^{ts1} and wild-type females. The TTX resistance of these heterozygous flies was found to be intermediate in value, being less than *shi*^{ts1} but greater than Oregon-R. This result correlates with the semi-dominant paralytic characteristics of *shi*^{ts}, in that *shi*^{ts}/+ heterozygotes are immobilised at 35° C but not at 29° C (L. E. Kelly, L. Hall, and D. T. Suzuki, in preparation).

It could be suggested that the resistance of *shi*^{ts} flies to TTX is simply a reflection of the difference in either their drinking behaviour or their ability to absorb the toxin through the gut wall. Consequently, the following experiment was performed. Bilateral mosaics were recovered from a cross of *shi*^{ts} males to females carrying an unstable ring X chromosome (*In(1)w^{sc}*)⁴. Loss of this ring X chromosome shortly after

TABLE 2 Effect of $5 \mu\text{g ml}^{-1}$ TTX on heterozygous *shi^{ts1}/+* females

Genotype	No. of flies		
	Dead	Alive	% kill
Oregon-R	14	212	93.8
<i>shi^{ts1}/shi^{ts1}</i>	176	63	26.3
<i>shi^{ts1}/+</i>	143	104	41.7

Controls were female. (The difference between the kill of *shi^{ts1}* females in this experiment and that shown in Fig. 1 is probably due to the use of a new batch of TTX for this experiment.)

fertilisation produces flies which contain cells which are heterozygous, *shi^{ts1}/+*, and hemizygous, *shi^{ts1}/0*, for the *shi^{ts1}* mutation. A mutation conferring yellow body colour was linked to the *shi^{ts1}*-bearing chromosome so that the genotype of the cells in the external chitin could be determined. Bilateral mosaics produced in this fashion have one side which is female and heterozygous for *shi^{ts1}* and the other side is yellow, male and genotypically *shi^{ts1}/0*. When shifted to 29°C , the *shi^{ts1}/0* half of the bilateral mosaic became paralysed, while the *shi^{ts1}/+* half remained normal. It has already been shown (Table 2) that *shi^{ts1}/+* flies are less resistant to TTX than hemizygous *shi^{ts1}/Y* flies. If, however, the resistance of *shi^{ts1}* flies to TTX was due solely to a reduction in the uptake of the drug, the haemolymph of the bilateral mosaic would be expected to contain a uniform distribution of TTX so that both halves of the animal would be equally affected. The bilateral mosaics were fed TTX at a concentration of $10 \mu\text{g ml}^{-1}$ for 10 h. These conditions had previously been found to severely debilitate a population of *shi^{ts1}/+* heterozygotes without causing much death. In the mosaics so treated, the *shi^{ts1}/+* sides of the flies were debilitated as expected; however, the *shi^{ts1}/0* halves of the same animals remained unaffected. Thus, the debilitation of the mosaics after TTX feeding was the reverse of the paralytic effects observed when the mosaics were shifted to 29°C . This result shows that the difference in resistance between *shi^{ts1}* and wild-type to TTX is not due to a difference in the uptake of the toxin; rather, the *shi^{ts1}* tissue is indeed resistant to the molecule.

Although the neurophysiological effect of TTX is known, there is still the possibility that the TTX-induced lethality in *Drosophila* is not due to a malfunctioning of the nervous system, but to some secondary toxic effect of the drug. To eliminate this latter possibility, it is necessary to show some TTX resistance of *shi^{ts1}* flies in neurophysiological terms. This was done by comparing the effect of a controlled dose of TTX on the electroretinogram (ERG) of *shi^{ts1}* and Oregon-R flies. TTX eliminates the 'on' and 'off' transients of the d.c. recorded ERG of wild-type *Drosophila* (Fig. 2). The heads of ten *shi^{ts1}* and ten Oregon-R flies were each injected with $0.1 \mu\text{l}$ of a $25 \mu\text{g ml}^{-1}$ solution of TTX in 0.6% NaCl and the ERGs were recorded. Within 5 min, nine out of the ten wild-type flies produced an ERG which did not possess the transients, while all ten of the *shi^{ts1}* flies retained the transients for longer than 30 min after the injection. While it cannot be said that each injected head retained all of the TTX, the result does indicate that those cells which give rise to these transients are more sensitive to TTX in Oregon-R flies as compared to the same cells in *shi^{ts1}* flies. It can be concluded from this experiment that the resistance to TTX which is conferred by the presence of the *shi^{ts1}* allele reflects an altered sensitivity of the nervous system to the toxin.

Since the *shi^{ts1}* alleles cause temperature-sensitive paralysis, it might be expected that the TTX resistance would also show a temperature dependence. As *shi^{ts1}* flies become paralysed when kept at temperatures in excess of 25°C for prolonged periods and as it has been found that 18°C rather

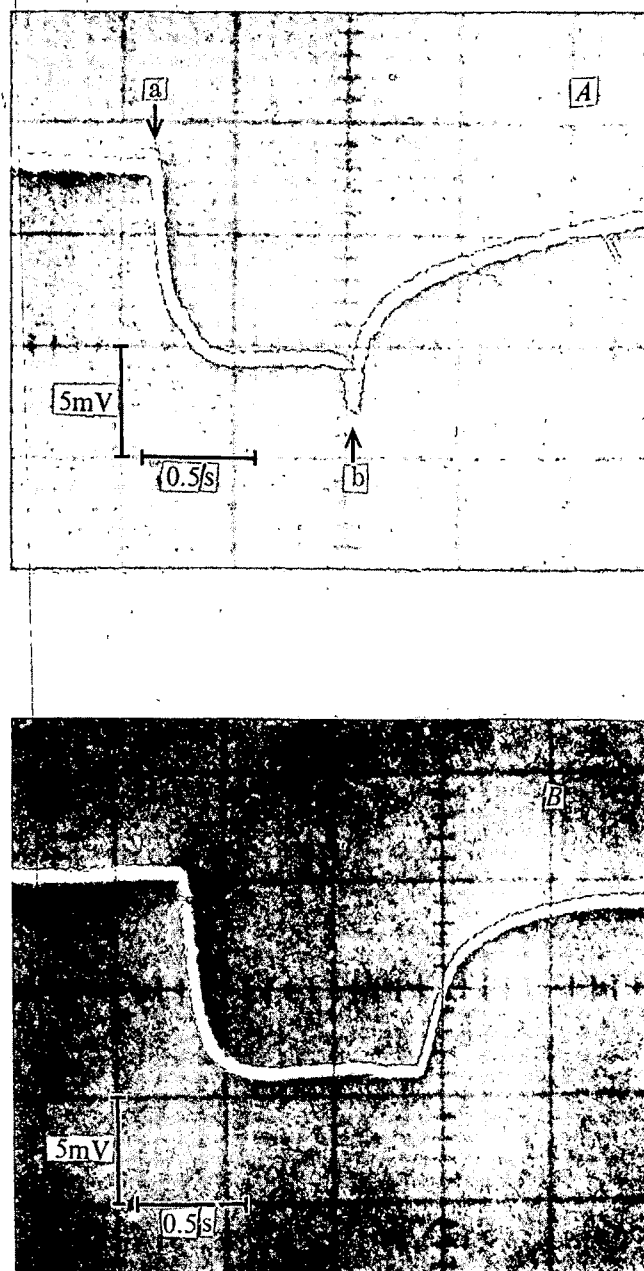


Fig. 2 Effect of TTX on the ERG of wild-type *Drosophila*. A, Normal ERG of a wild-type fly. Note the presence of the 'on' transient (a) and the 'off' transient (b). B, ERG from a wild-type fly injected with $0.01 \mu\text{l}$ of a $100 \mu\text{g ml}^{-1}$ solution of TTX in 0.6% NaCl. Note the loss of the transients.

than 22°C is the true permissive temperature for *shi^{ts1}* (L. E. Kelly, L. Hall, and D. T. Suzuki, in preparation), the resistance of *shi^{ts1}* to TTX at 17°C and 22°C was compared. Kill curves were constructed for *shi^{ts1}* and Oregon-R at 17°C . As both strains seemed however to be more resistant to TTX at this temperature, the incubation time was extended to 48 h. This produced a kill curve for Oregon-R which is comparable to that obtained at 22°C . The curves obtained (Fig. 3) show that there is a large increase in the resistance of *shi^{ts1}* to TTX at this temperature, the LD_{50} of *shi^{ts1}* being some seven times greater than wild type. Preliminary investigations of *shi^{ts1}* flies at 22°C and 25°C indicate that *shi^{ts1}* is also more resistant at the lower temperature. Finally, if the *shi^{ts1}* flies are fed TTX at 22°C to the point of debilitation and then shifted down to 17°C , they recover from the debilitation

within 5 min, while no comparable effect is observed with Oregon-R flies. The converse is also true; *shi¹¹¹* flies which are debilitated by TTX at 17° C are immediately paralysed and die when shifted to 22° C. This indicates two things; first, the debilitation induced by TTX is temperature reversible in *shi¹¹¹* and secondly, the increased resistance of Oregon-R and, in part, of *shi¹¹¹* at 17° C is probably due to a reduced rate of uptake of liquid in both strains. This may be a consequence of reduced dehydration at the lower temperature.

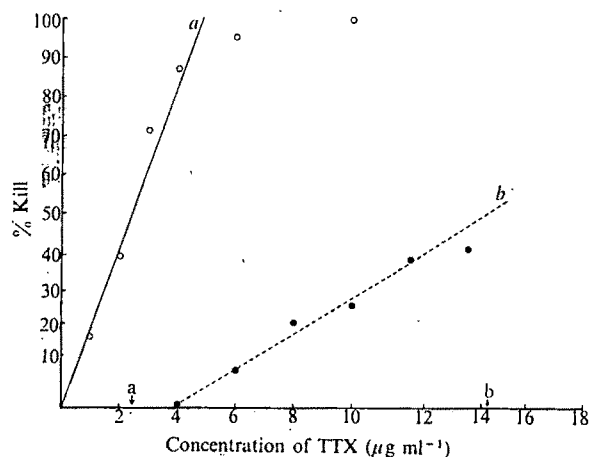


Fig. 3 Effect of increasing concentrations of TTX on a, Oregon-R, and b, *shi¹¹¹* flies at 17° C. Incubation time was 48 h. Arrows marked a and b indicate LD₅₀ for Oregon-R and *shi¹¹¹* flies respectively.

In conclusion, it has been shown that the *shi¹¹¹* alleles confer on *Drosophila* a resistance to TTX which, like the paralysis of these flies, is temperature dependent. It has also been demonstrated that this resistance is attributable to an alteration in the properties of the nervous system. The TTX resistance results obtained for the *shi¹¹¹* and *shi¹¹²* alleles are interpreted as resulting from alterations of different sites in the protein molecule encoded by this gene. It is suggested that the *shi¹¹¹* locus is involved in the production of some component of the regenerative sodium channel and that the presence of the *shi¹¹¹* allele, as well as producing temperature-sensitive paralysis, also reduces the affinity of this channel for TTX.

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Dinosaur Monophyly and a New Class of Vertebrates

TRADITIONALLY dinosaurs are classified as two or three separate, independent groups of reptiles in the Subclass Archosauria. But evidence from bone histology, locomotor dynamics, and predator/prey ratios strongly suggest that dinosaurs were endotherms with high aerobic exercise metabolism, physiologically much more like birds and cursorial mammals than any living reptiles¹⁻⁸. Recently Ostrom has argued forcefully that birds are direct descendants of dinosaurs and inherited high exercise metabolism from dinosaurs^{1,8}. Here we present evidence that dinosaurs are a single, monophyletic group, and that the key advancements of endothermy and high exercise metabolism are justification for removing dinosaurs from the Reptilia and placing them with birds in a new class, the Dinosauria.

The two generally accepted orders of dinosaurs, the Saurischia and Ornithischia, are usually interpreted as independent derivatives of primitive thecodontian reptiles of the Triassic, but all known Triassic dinosaurs can be distinguished from typical thecodontians (Fig. 1)⁹⁻²³.

Most thecodontians had a wide-track sprawling gait or a crocodile-like semi-erect gait, both requiring much multidirectional mobility at the shoulder⁶. The thecodontian glenoid, or shoulder socket, was lizard or crocodile-like—being a saddle-shaped notch facing outward and backward, permitting humerus rotation, abduction, and backswing. Dinosaur glenoids were concave sockets facing mostly down and backwards, little outwards, restricting humerus movement severely to a fore-and-aft vertical plane—the 'fully erect gait'⁶.

Also, in thecodontians and reptiles, generally, the deltopectoral crest (*dp* in Fig. 1) is located close to the humerus head, giving the pectoralis musculature good leverage for rotating the humerus about its long axis but relatively little leverage for a vertical humerus backswing. In dinosaurs, the vertical backswing leverage was increased by moving the deltopectoral crest down the humerus shaft (Figs. 1 and 2).

In thecodontians and reptiles generally, the fourth trochanter (*4t* in Fig. 1) usually was located close to the femoral head, giving the caudifemoralis brevis musculature good long-axis rotational leverage, but little leverage for a vertical backswing. In dinosaurs, the apex of the fourth trochanter was developed into a distally directed flange, increasing the leverage for a vertical backswing (Figs 1 and 2).

A sprawling or semi-erect gait directs the thrust of the femur strongly inwards into the acetabulum, or hip socket. The thecodontian acetabulum usually was a strong, continuous bony surface as in most living reptiles. In dinosaurs, femoral action was more restricted to a vertical plane; the acetabulum was perforated to allow deeper penetration of the inturned femoral head, and the weight of the body was transmitted from the proximal surface of the femur to the strong dorsal rim of the acetabulum²⁴.

The thecodontian hand, known in phytosaurs²⁰, aetosaurs^{17,25}, rauisuchids²⁶ and 'Cheirotherium'^{27,28}, was crocodile-like with five long digits, the inner three subparallel, the outer two divergent (Fig. 1). The hand of Triassic saurischian dinosaurs is distinctive (Fig. 1) with a very short, stout metacarpal I; a long, strong thumb phalanx I; a powerful, curved, trenchant thumb claw; thumb articulations forcing the claw to diverge and point inwards during extension, converge with digits II and III and point downwards during flexion; long digits II and III, subequal and subparallel, bearing trenchant claws; and reduced digits IV and V²⁹. The hand of the better known primitive ornithischians (fabrosaurids and hypsilophodontids) differed from that of Triassic saurischians in lacking the long thumb and having blunt hooves instead of claws on digits I to III (ref. 30). Recently, a complete articulated skeleton of *Heterodontosaurus*, a Triassic ornithischian, was collected by A. W. Crompton, now at Harvard, for the South African Museum. The hand is virtually identical to that of

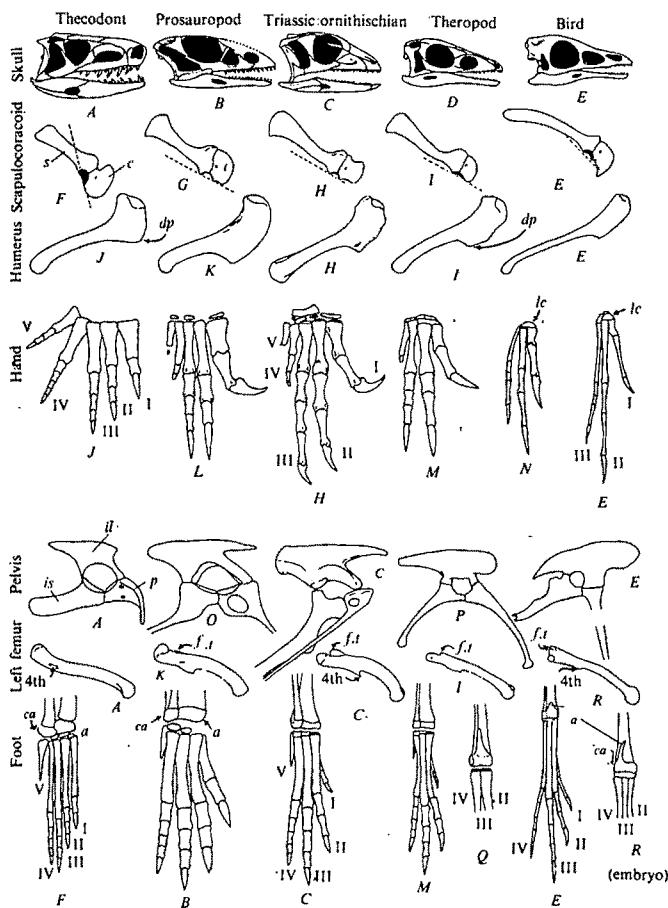


Fig. 1 Skulls, forelimbs and hindlimbs of thecodontians, dinosaurs and birds. All views of right elements except femur. (A) *Euparkeria*, (B) *Anchisaurus*, (C) *Fabrosaurus*, (D) *Compsognathus*, (E) *Archaeopteryx*, (F) *Gracilisuchus*, (G) *Plateosaurus*, (H) *Heterodontosaurus*, (I) *Halticosaurus*, (J) *Ticinosuchus*, (K) *Efraasia*³⁹, (L) *Thecodontosaurus*, (M) *Syntarsus*, (N) *Deinonychus*, (O) *Ammosaurus*, (P) *Coelophysis*, (Q) an ornithomimid and (R) *Grus*. *Anchisaurus*, *Fabrosaurus* (except skull), *Heterodontosaurus*, *Efraasia* and *Thecodontosaurus* from original material; data for the others from refs 20, 21, 26, 28, 36, 44, 53–55. (s) Scapula, (c) coracoid, (dp) deltopectoral crest, (lc) lunate carpal, (il) ilium, (is) ischium, (p) pubis, (a) astragalus, (ca) calcaneum, (ft) femorotibialis, (4t) fourth trochanter, (---) long axis of glenoid.

Triassic saurischians in all six of the features cited above. Although specialised cranially³¹, *Heterodontosaurus* probably represents the original ornithischian hand pattern, inherited from saurischians, where the thumb was specialised for defence and digits II and III were used for defence, digging, and support during slow, quadrupedal locomotion. In more advanced ornithischian hands, the defensive function was lost, the thumb was secondarily shortened and simplified, and the claws were replaced by hooves, but in *Hypsilophodon*, digits II and III are still subequal and subparallel³⁰.

The origin of the femorotibialis, a knee extensor, in most thecodontians and in reptiles generally, is on the smooth anteriolateral surface of the femoral shaft. In primitive saurischians and ornithischians, the origin was expanded by the development of a spike-like ridge (*f.t.* in Fig. 1). In advanced saurischians and ornithischians, and in birds, this spike becomes a large crest—the 'lesser trochanter', not homologous to the lesser trochanter of mammals. Increased muscle power for knee extension probably was related to the development of the fully erect gait. Ornithomimid thecodonts have this crest but differ from dinosaurs dramatically at the ankle and forelimb^{10,18}.

The ankle of most thecodontians was complex and crocodile-like: the astragalus articulated movably with the calcaneum and tibia, and the calcaneum bore a 'heel' (calcaneal tuber) for the gastrocnemius musculature^{24,32}. In advanced thecodontians the astragalus-calcaneal joint was a deep ball and socket³³. In sharp contrast, the dinosaur ankle was stiff, simple and bird-like: the astragalus and calcaneum were rounded caps fixed immovably on the ends of the tibia and fibula; the distal tarsals were immovable caps on the metatarsal heads, and the tarsal joint was a simple unidirectional hinge between astragalus-calcaneum and distant tarsals (Fig. 1)^{24,28}. Saurischian diphyly has been suggested because some theropods, such as *Allosaurus*, have deep ilia and a thin, anterior ascending process of the astragalus, while prosauropods have unexpanded ilia, no ascending process, and a peg-in-notch astragalus-tibial joint^{22,34}. Triassic forms, however, show how the transition from prosauropod-grade to advanced theropod grade saurischian occurred. The Triassic theropods *Syntarsus* and *Halticosaurus* had expanded ilia but retained a prosauropod-type astragalus-tibial joint without an anterior ascending process^{35,36}. In general, the prosauropod grade of postcranial anatomy seems to be the primitive dinosaur pattern. Details of tarsal structure are often obscured by incomplete ossification, but the pattern in *Syntarsus* is virtually identical to that of *Heterodontosaurus* (Fig. 1). Digit I arises two-thirds down the length of metatarsal II and is reduced and slightly divergent; digits II and IV are subequal; digit III is the longest; digit V is reduced to a splint; distal tarsal IV is cuboid; III is rectangular; II is a thin plate and the astragalus-calcaneum are prosauropod-like.

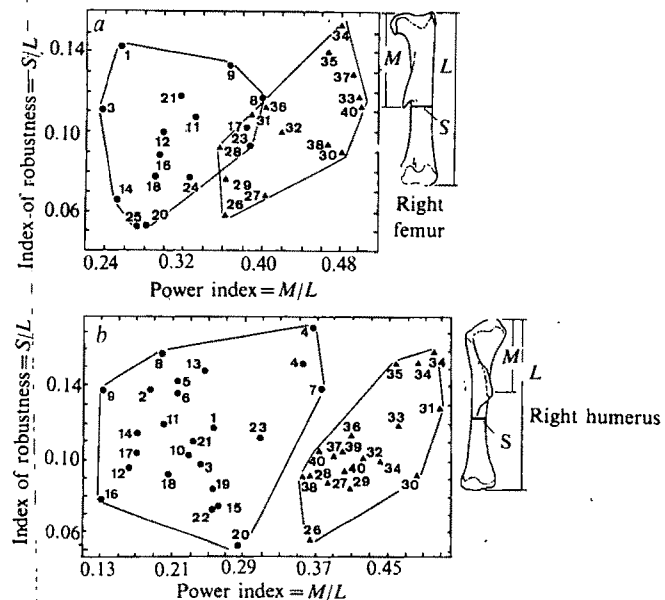


Fig. 2 Robustness and position of muscle attachments, (a) in the femur and, (b) in the humerus. The data are taken from specimens and refs 9, 10, 13, 15–20, 26, 28, 30, 36, 38, 39, 42, 43. Thecodonts, ●, are (1) *Proterosuchus*, (2) *Erythrosuchus*, (3) *Euparkeria*, (4) *Shanisuchus*, (5) *Fenhsuchus*, (6) *Wangisuchus*, (7) *Argentinosuchus*, (8) *Neoargosaurus*, (9) *Stagonolepis*, (10) *Palaeorhinus*, (11) *Phytosaurus*, (12) *Rutiodon*, (13) *Myristosuchus*, (14) *Gracilisuchus*, (15) *Turfanosuchus*, (16) *Ornithosuchus*, (17) *Riojasuchus*, (18) *Ticinosuchus*, (19) *Sphenosuchus*, (20) *Pseudhesperosuchus*, (21) *Pedeticosaurus*, (22) *Triassolestes*, (23) *Alligator*, (24) *Lagosuchus*, and (25) *Lagerpeton*. Dinosaurs, ▲, are (26) *Staurikosaurus*, (27) *Ischisaurus*, (28) *Herrerasaurus*, (29) *Halticosaurus*, (30) *Efraasia*, (31) *Massospondylus*, (32) *Teratosaurus*, (33) *Plateosaurus*, (34) *Riojasaurus*, (35) *Melanorosaurus*, (36) *Anchisaurus*, (37) *Fabrosaurus*, (38) *Heterodontosaurus*, (39) *Dysalotosaurus*, and (40) *Hypsilophodon*. (S) Minimum shaft diameter, (L) length of femur or humerus, (M) distance from head to muscle attachment (deltopectoral crest in humerus, fourth trochanter in femur).

The radiations of thecodontians and dinosaurs were based on different trends in locomotor anatomy, behaviour, and exercise metabolism. The earliest thecodontians, the proterosaurs¹⁵, had short limbs and a sprawling posture with a wide range of movement at each joint⁶. Stride length and power in the sprawling gait are increased by vertebral column undulations, and thecodontians generally lack adaptations for inhibiting lateral undulation. The complex ankle of advanced thecodontians was probably a response to selection for finer control of supination-pronation and greater leverage in sprawling locomotion. Thecodontian footprints²⁷ indicate that digit V in the hand and foot were strongly divergent and powerfully muscled, like digit I of climbing mammals. Flexibility at the major limb joints plus divergent digit V probably gave thecodontians a wide locomotor repertoire, including some digging, climbing, and movement over uneven ground. Some of the later Triassic thecodontians (such as aetosaurs and phytosaurs⁶) retained the sprawling gait and others, such as rauisuchids and ornithosuchids^{10,18}, developed a crocodile-like locomotor system which make possible both sprawling and semierect gaits^{6,37}. Inturned femoral heads indicate that a few thecodontians such as *Hallopus* and some advanced rauisuchids and ornithosuchids¹⁹, may have had narrow-tracked, fully erect hindlimbs, but even these forms retained the complex ankle.

The first well-known dinosaur is *Staurikosaurus*, of late Middle or early Late Triassic^{38,39}, a long-jawed predator with very long, gracile hindlimbs and postcranial anatomy of prosauropod grade (the femoral head is not as sharply inturned as in later dinosaurs and there is an oval acetabulum). More advanced theropods, such as *Ischisaurus* and *Herrerasaurus*, and ornithischians, such as *Pisanosaurus*, appear shortly after *Staurikosaurus*⁹. The original dinosaur stock were probably predators with higher running speeds and exercise metabolism than in thecodontians of comparable size. Early dinosaurs may have been dipedal at top speed, but the strong forelimb modifications for the fully erect gait and the prosauropod-like hand indicate that quadrupedal progression was very important. Even at the prosauropod grade, dinosaur bone histology was identical to that of endothermic mammals⁴. Restriction of joint movement to a fore and aft, vertical plane and the stiff, bird-like ankle would have made climbing difficult for any dinosaur⁴⁰. The vertebral column of early dinosaurs was stiffened to inhibit lateral undulations by the development of extra vertebral articulations, the hyposphene-hypantra in saurischians, and long ossified tendons appressed against the neural spines in ornithischians. The locomotor repertoire of dinosaurs was narrow, rather restricted to moving over fairly even terrain. Recent experiments⁴¹ show that erect locomotion does not require less energy for a given speed than sprawling, as has usually been assumed⁶, but erect locomotion probably does increase manoeuvrability at very high speeds. The dinosaur radiation was based on a concentration of behavioural, physiological, and anatomical adaptations for high, sustained running speeds which made them irresistible predators and competitors to contemporary thecodontians and the larger mammal-like reptiles.

Could the similar postcranial adaptations of Triassic dinosaurs reflect merely convergent evolution of the erect gait from different thecodontians? We believe that the answer to this question is almost certainly no. Evolution of erect posture does not invariably lead to an avian-dinosaur type joint pattern. Some thecodonts and many mammals, such as carnivores and ungulates, have a fully erect gait but retain complex ankles with astragalar-calcaneal mobility and calcaneal 'heels'. The very detailed similarity in all the major joints, especially the hands and feet, of Triassic ornithischians and saurischians makes dinosaur polyphyly exceedingly unlikely. Moreover, the pattern of increasing dinosaur diversity in the Triassic upwards from the stratigraphic level of *Staurikosaurus* suggests one monophyletic radiation. Some of the little 'rabbit thecodontians' such as *Lagosuchus* and *Lagerpeton*^{42,43}, have im-

mobile astragalar-calcaneal joints and lack calcaneal 'heels', and may be related in some way to dinosaur origins.

The prosauropod saurischians have a mixture of primitive and advanced features which show how ornithischians evolved from saurischians (Fig. 1). In the primitive ornithischian dentition, represented by *Fabrosaurus*, the anterior teeth were non-serrated, pointed and slightly incurved with swollen bases and the cheek teeth were serrated with triangular crowns⁴⁴. In some prosauropods, such as *Massospondylus* and probably *Anchisaurus*, the basic pattern was similar: anterior, simple teeth and triangular, serrated cheek teeth. In advanced prosauropods, such as *Plateosaurus* and in most ornithischians, the jaw articulation was depressed below the tooth row level, but in *Fabrosaurus* and *Anchisaurus* the articulation was still on the same level as the tooth row⁴⁴. The lower jaw of most ornithischians was deep and massive, but the fabrosaur jaw was slender like that of *Anchisaurus*. In early ornithischians the origin of iliotibialis, a knee extensor co-inserting with the femorotibialis, was expanded by the development of a long anterior iliac prong. Such a prong is unknown in thecodontians and most prosauropods, but is present in very fabrosaur-like configuration in *Anchisaurus* and *Ammosaurus*⁴⁵. In ornithischians the primitively broad, plate-like ventral surface of the pubis and ischium were deeply excavated and reduced; such excavations had begun in the prosauropods *Anchisaurus* and *Ammosaurus*⁴⁵.

The prosauropod grade is quite primitive, and herbivorous prosauropods must have diverged very early from the basal, predatory dinosaur stock. It should be noted that no thecodontians show trends away from carnivory towards herbivory, except the aetosaurs, sprawling, armoured types totally unlike ornithischians¹⁷. Small prosauropods with *Anchisaurus*-type jaw articulations, ilia and pubes were probably the immediate ancestors of ornithischians. The adaptive shift probably was an emphasis on ultra-high speed bipedality plus adaptations for prehension and cutting of plant fibres. Excellent fabrosaur material collected for the South African Museum and now at Harvard, shows that the development of an upper beak had just barely begun in *Fabrosaurus*. The fabrosaur predentary was small, sharply pointed, and movably articulated with the dentary; the dentary symphysis was mobile. The predentary beak could serve as a gouge for digging into plant stems and as a cutting edge occluding with the arcade of premaxillary teeth, much like the incisors of kangaroos⁴⁶, while the mobile symphysis permitted the retention of independent control of each jaw ramus, important for tooth-to-tooth shearing on one side of the mouth at a time. Excavations in the maxilla and dentary, for cheek pouches to retain the cut ends of plant material⁴⁷, had begun in *Fabrosaurus*. All known Triassic ornithischians had very long hind limbs with elongate distal extremities indicating very high speeds. A short trunk facilitates the maintenance of balance in a highly bipedal animal, but herbivory demands a long, bulky gut. Ornithischians solved the balance problem by rotating the pubis posteriorly so that the distal end, marking the end of the gut, lay far posterior to the acetabulum. Thus, the gut was extended between the hindlimbs and the pre-acetabular trunk section could be abbreviated⁴⁸.

Although Walker⁴⁹ has cited some resemblances between living birds and crocodile-like thecodontians (sphenosuchids), we believe that Ostrom has shown that the similarities between *Archaeopteryx* and small theropods are so detailed and comprehensive that the immediate ancestor of birds must have been a saurischian dinosaur^{1,8,50}. Some of the features cited by Walker as evidence of crocodile-bird relationships are actually widespread among tetrapods: cranial salt glands are developed in lizards, especially in *Amblyrhynchus*⁵¹; forearm articulations which force the radius distally during forearm flexion are present in salamanders, *Sphenodon*, and lizards. Although details are obscure, the *Archaeopteryx* skull seems much more like the light, kinetic skulls of small theropods than the solidly braced sphenosuchid structure⁵². *Archaeopteryx*

has the complete suite of seven features listed above as diagnostic of the primitive dinosaur erect gait. In flying birds, bats and pterosaurs the glenoid faces strongly outwards to make possible the abduction of the humerus necessary for gliding and powered flight. Significantly, *Archaeopteryx* retained the typical, downward-facing dinosaur glenoid; it is difficult to imagine how this joint would have made either flight or gliding possible. The initial evolution of feathers probably was not associated with flight, a hypothesis developed at length by Ostrom¹.

Ostrom has shown that the hands and feet of *Archaeopteryx* are identical in detail to certain Jurassic/Cretaceous theropods, and differ only in being larger relative to the body size (Fig. 3)^{1,50,53-55}. Feathers may have been widespread in bird-like theropods. *Archaeopteryx* strongly resembles small theropods in general body plan with a short trunk, thin, flexible neck, very long hindlimbs and long fingers bearing trenchant claws for snagging prey. Sphenosuchid thecodontians were quite different (Fig. 3) with a long trunk, thick neck and long forelimbs with short digits adapted for running and not prehension.

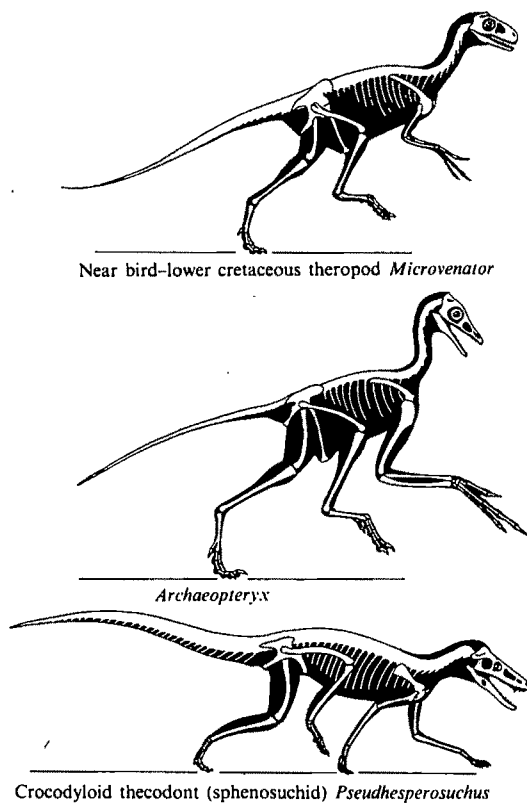


Fig. 3 Body form in a small theropod dinosaur, the first known bird and a crocodyloid thecodontian. *Microvenator* hand and skull restored after *Ornitholestes*. Data from refs 10, 53-55.

Endothermy and high aerobic exercise metabolism are sufficient justification for separating birds into a class distinct from other living sauropsid tetrapods. But endothermy and high exercise metabolism were probably already present in the dinosaur ancestors of birds and are the key features differentiating dinosaurs from crocodylians and the other extinct archosaurs. The bird radiation has produced many species, but the structural diversity is not more striking than that within the Ornithischia or Saurischia. Bird physiology is also rather stereotyped. The avian radiation is an aerial exploitation of basic dinosaur physiology and structure, much as the bat radiation is an aerial exploitation of basic, primitive mammal

physiology. Bats are not separated into an independent class merely because they fly. We believe that neither flight nor the species diversity of birds merits separation from dinosaurs on a class level. Among all amniotes, the most profound adaptive shift was from ectothermy to endothermy, which occurred during the origin of mammals and dinosaurs. Therefore we propose the erection of a Class Dinosauria, to include as subclasses the Saurischia, Aves and Ornithischia. The currently recognised suborders of dinosaurs would be elevated to orders (Fig. 4). Thecodontians, crocodylians, and pterosaurids

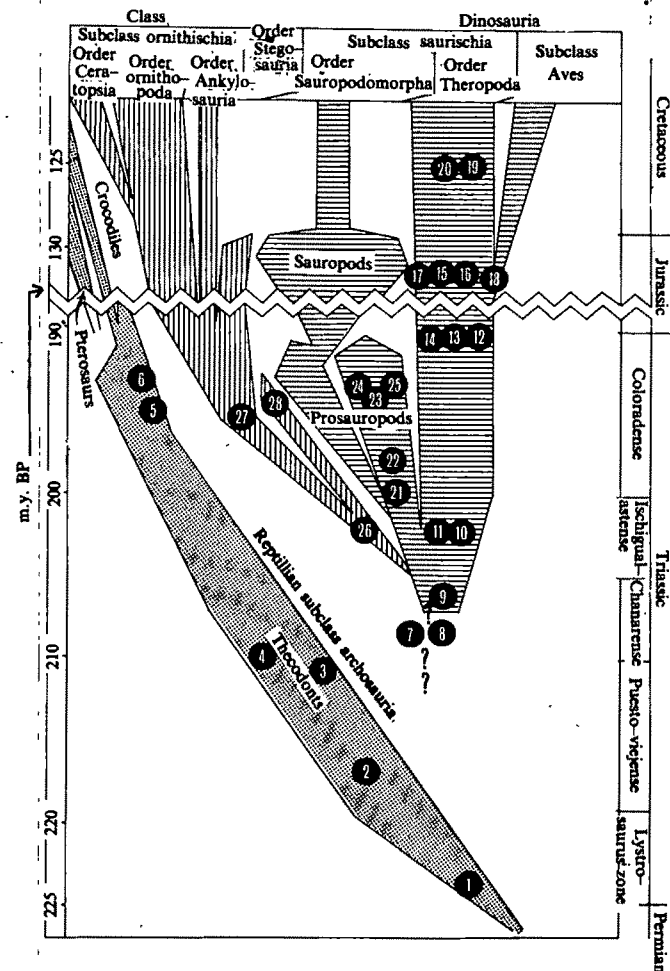


Fig. 4 Suggested phylogeny and classification of dinosaurs. Position of *Lagosuchus* and *Lagerpeton* is very uncertain. Within orders, ancestor-descendent relationship is not necessarily indicated by stratigraphic level of known specimens, for example, *Pisanosaurus* is earlier than, but in some ways more advanced than the earliest known *Fabrosaurus*. (1) *Proterosuchus*, (2) *Euparkeria*, (3) *Ticinosuchus*, (4) *Gracilisuchus*, (5) *Sphenosuchus*, (6) *Pseudhesperosuchus*, (7) *Lagerpeton*, (8) *Lagosuchus*, (9) *Staurikosaurus*, (10) *Ischisaurus*, (11) *Herrerasaurus*, (12) *Coelophysis*, (13) *Syntarsus*, (14) *Halticosaurus*, (15) *Coelurus*, (16) *Ornitholestes*, (17) *Compsognathus*, (18) *Archaeopteryx*, (19) *Microvenator*, (20) *Deinonychus*, (21) *Thecodontosaurus*, (22) *Efraasia*, (23) *Plateosaurus*, (24) *Ammosaurus*, (25) *Anchisaurus*, (26) *Pisanosaurus*, (27) *Fabrosaurus* and (28) *Heterodontosaurus*.

would remain in the reptilian subclass Archosauria, which would stand to Dinosauria much as the reptilian subclass Synapsida (the mammal-like reptiles) stands to Mammalia. This new classification, we believe, reflects more faithfully the major evolutionary steps. Ectotherms and forms transitional to endotherms are retained in the Reptilia and the two highly successful endothermic groups, mammals and dinosaurs, are given separate class status.

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Viroids and viral hepatitis in marmosets

DIENER¹ and Zuckerman² have recently speculated that hepatitis A and B, respectively, may be caused by infective naked nucleic acids or viroids. In response to their hypotheses, we felt it would be of interest to investigate the nature of an agent known to cause hepatitis in marmoset monkeys. We reasoned that if the aetiological agent of hepatitis found in serum is a free, or naked, nucleic acid in this system, then its infectivity should be destroyed by the action of a specific endonuclease. For example, a free viral DNA would be rendered uninfected after treatment with pancreatic DNase. The virus used in our study was the 'Barker' agent, originally recovered by Deinhardt *et al.* from acute phase sera of marmosets inoculated with serum from a human case of viral hepatitis³. This virus consistently induces hepatitis in such animals, although its pedigree as a human hepatitis agent has been a subject of controversy⁴.

As many animal sera contain RNase activity, but not DNase activity, we assumed the postulated viroid for Barker-induced marmoset hepatitis, if it existed, would most likely be a naked DNA. To test this assumption, we divided a pool of infective acute-phase marmoset serum into two equal aliquots. One aliquot was treated with pancreatic DNase (EC 3.1.4.5) at 20 µg ml⁻¹ for 1 h at 37° C; the other aliquot was heated to 37° C for 1 h without any other treatment. Three *S. nigricollis* marmosets were inoculated intravenously (i.v.) with treated serum (0.25 ml each); three other *S. nigricollis* marmosets were inoculated i.v. with untreated serum. The course of infection in each marmoset was monitored by measuring serum glutamic pyruvic transaminase (SGPT) and serum isocitric dehydrogenase (SICD) activities as indicator enzymes for liver damage.

All six marmosets demonstrated elevated SGPT and SICD activities by the fourth or fifth week after inoculation, indicating liver damages had been sustained in each animal. Clearly, then, the marmoset hepatitis agent cannot be a DNA viroid as all three animals receiving DNase treated serum showed convincing enzymatic evidence of viral hepatitis. The results of our preliminary study are consistent with the tentative finding by Deinhardt *et al.*⁵ that the Barker hepatitis agent bands in CsCl at a buoyant density of 1.2. Viruses usually band around $\rho = 1.2-1.4$, whereas free viral DNA bands at about $\rho = 1.7$.

While the possibility still exists that the marmoset hepatitis agent is another type of viroid, that is, a unique double-stranded RNA (or RNA:DNA hybrid)-protein conjugate which bands in CsCl at $\rho = 1.2$, our experimental evidence at least rules out the presence of a simple DNA viroid in serum containing the marmoset agent.

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Nutrient transfer between the seagrass *Zostera marina* and its epiphytes

THE leaves of seagrasses often harbour dense populations of macroscopic and microscopic algal epiphytes. In many cases seagrasses grow in waters that are extremely low in dissolved inorganic nutrients yet they have high epiphyte standing stocks. These field observations led to direct experiments on the transfer of nitrogen and carbon from the dissolved nutrient pool in the interstitial waters of the sediments into the root system of eelgrass, *Zostera marina*, and through the plant to the algae on its leaves. Previous studies^{1,2} on seagrasses have shown that the high productivity of seagrass meadows is largely maintained by the nutrient pool of the sediments. Since the plant system leaks phosphorus and, presumably, other nutrients, it is possible that the production of the leaf epiphytes is indirectly sustained by the nutrients in the sediments. This mechanism has been suggested by several workers for marine and freshwater macrophytes³⁻⁷ but definitive experiments are lacking. Here we report the results of experiments designed to test this hypothesis.

Eelgrass, *Zostera marina* L., was collected from beds on Visovodof Island in the Aleutian Islands of Alaska, on August 6, 1972, a few hours before experiments began. Sediments were removed from the roots, and then plants were placed in partitioned plexiglass containers in which the water surrounding the leaves was isolated from that surrounding the roots and rhizomes (Fig. 1). Each experimental container held three plants with their natural epiphyte populations. Containers were filled with 3 l of filtered seawater in the upper level and 1.5 l in the lower. Since the plants grow in anoxic sediments, the lower water surrounding the roots and rhizomes was stripped of dissolved oxygen by bubbling with a helium-carbon dioxide gas mixture before the experiment. The seal round the plant between water levels was maintained by a septum stopper fitted to the lower stem and sealed by silicone stopcock grease.

We added $^{15}\text{N-NH}_4^+$, $^{15}\text{N-NO}_3^-$, or $^{15}\text{N-(NH}_2)_2\text{CO}$ to the lower compartment of each container in low (33 $\mu\text{gatom N l}^{-1}$) or high (67 $\mu\text{gatom N l}^{-1}$) concentration. In addition, 20 μCi of ^{14}C -labelled HCO_3^- was also added to the lower compartment of each container. The experimental containers were incubated for 8 h under natural light and ambient temperatures (about 12° C). The lower compartment of all containers was darkened.

At the end of incubation the plants were removed from a container, one was bisected into leaves and roots-rhizomes and frozen immediately, and the other two plants were dissected into leaves, stem, and roots-rhizomes and dried at 60° C for 24 h. The epiphyte community of a leaf was

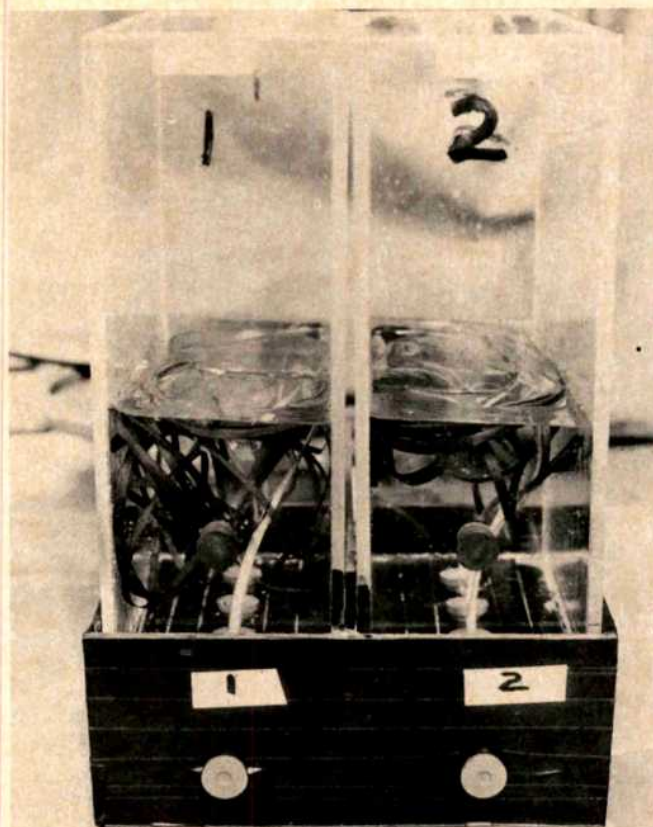


FIG. 1 Partitioned container with *Zostera* for measuring uptake of carbon and nitrogen. Roots and rhizomes are in the lower blackened compartment separated at leaf base by septum stoppers; only the portion of the plant above the septum stopper is visible.

sampled by wiping the leaf on a glass slide and washing the algae removed on to a silver filter. After filtering, the epiphyte samples were dried at 60° C for 24 h and retained in a desiccator until analysed first for ^{14}C and then for ^{15}N content. The epiphytes comprised mainly several species of diatoms.

Carbon uptake by *Zostera* was measured by converting organic carbon to CO_2 by combustion and the radioactivity of the $^{14}\text{CO}_2$ determined in a liquid scintillation counter after the method suggested by Wetzel⁸ for aquatic plants. The ^{14}C content of the epiphytes was determined by Geiger-Mueller counting in a procedure similar to that for phytoplankton productivity⁹.

Nitrogen uptake was measured by determining the quantity of ^{15}N in the plant and epiphyte material. The organic

TABLE 1 Carbon and nitrogen uptake by roots of *Zostera marina* L. and transport through the plant to leaf epiphytes. Carbon values are single observations; nitrogen values are means of two observations.

	Carbon uptake ($\mu\text{g g}^{-1} \text{h}^{-1}$)				Nitrogen uptake (10^2 % uptake h^{-1})			
	Roots and rhizomes	Stems	Leaves	Leaf epiphytes*	Roots and rhizomes	Stem	Leaves	Leaf epiphytes
Experiment I ^{15}N added as NH_4^+								
Low [N]	2.69	1.76	0.07	(0.03)	3.35	0.39	0.32	7.8
High [N]	11.83	0.27	0.13	(0.01)	4.16	1.01	0.25	7.2
Experiment II ^{15}N added as NO_3^-								
Low [N]	1.23	1.58	0.17	(0.01)	1.32	1.32	1.50	21.9
High [N]	5.91	0.24	0.08	(0.04)	1.82	1.33	1.88	6.5
Experiment III ^{15}N added as $(\text{NH}_2)_2\text{CO}$								
Low [N]	—NO DATA TAKEN—				0.025	0.033	0.051	8.7
High [N]					0.031	0.038	0.048	7.3

* Units for carbon uptake by epiphytes are μg per g leaf per hour.

Low [N] = 33 $\mu\text{gatom N l}^{-1}$.

High [N] = 67 $\mu\text{gatom N l}^{-1}$.

nitrogen was converted to N_2 by an automated Dumas technique¹⁰ and the ^{15}N content of the N_2 subsequently determined in a Bendix mass spectrometer. Uptake calculations followed the procedure of Dugdale and Goering¹¹. The data are presented as % uptake, and the relative velocity of nitrogen uptake; absolute uptake is obtained by multiplying the latter by the nitrogen content of the material.

The results of all experiments indicate a direct transfer of carbon and nitrogen from *Zostera* to the epiphytes on its leaves. ^{14}C labelled HCO_3^- and ^{15}N labelled NO_3^- , NH_4^+ , and $(NH_2)_2CO$ were all absorbed from solution by the root-rhizome system of the plant and the labels were subsequently transported to all parts of the plant and to the epiphytes on the leaves (Table 1). In all experiments after 8 h the ^{15}N content was larger in the epiphytes than in any part of the plant. Within a plant, a ^{15}N concentration gradient was observed with greatest amounts in the root-rhizome system and least in the leaves. The distribution of ^{14}C was generally similar to that of ^{15}N .

These results are positive evidence for the transfer of nitrogen from the sediments to the epiphyte community of a rooted macrophyte and could well be part of the explanation for how high standing stocks of epiphytic algae are maintained in waters with low concentrations of dissolved inorganic nutrients. It is possible that nitrogen and carbon are transferred directly from *Zostera* to the epiphytes as organic compounds, but transfer probably occurs by leakage of organic or inorganic compounds from the plant and subsequent uptake by the algae.

Previous experiments have shown that phosphorus absorbed by the root-rhizome system was in part lost across the leaves, so it is likely that this nutrient is also available for epiphyte growth. Harlin⁴ has shown that ^{14}C and ^{32}P -PO₄ could be transferred from *Zostera* to the algal epiphyte *Smithora naiadum*. In addition, Brylinsky³ has shown that several species of tropical seagrasses lose about 2% of the carbon fixed in photosynthesis across the leaves; the loss was in the form of low molecular weight organic compounds. The excretion of organic carbon and nitrogen has been measured in rooted freshwater macrophytes⁵⁻⁷.

It is likely that *Zostera* and most other seagrasses obtain their nutrients from the sediments through the roots. There is also evidence that some inorganic carbon is taken up by the roots and transported to a site where it can be fixed in photosynthesis. This mechanism has been found in some species of vascular plants in freshwaters¹².

Our work indicates a symbiotic relationship between seagrasses and their epiphyte community. This implies complexities in nutrient and productivity cycles in shallow waters dominated by rooted vascular macrophytes. Clearly, epiphyte productivity is enmeshed with that of its associated macrophyte.

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Why kangaroos hop

KANGAROOS (family Macropodidae, order Marsupialia) are the only known group of large Australian herbivorous marsupials ever to have evolved major cursorial specialisations. During fast locomotion macropodids progress by a ricochet (bipedal saltation) involving a series of rebounds in which the two hind feet strike the ground at the same moment or practically synchronously and the forefeet not at all¹. In macropodids the axis of the hind foot is through digit IV which is unique among large cursorial mammals; perissodactyls and most rodents have the axis through digit III, while in artiodactyls, carnivores and leporids it lies between digit III and IV². These differences are the result of preadaptive complexes in the immediate ancestors of these groups.

The earliest placentals were probably terrestrial³ and the pes was primitively pentadactylous with a nonserial tarsus^{4,5}. Two basic vectors of weight transfer from the tibia to the digits were involved (Fig. 1A), one through the astragalus-navicular-cuneiforms-digits I-III, and the other through the astragalus-calcaneum-cuboid-digits IV-V. The non-serial tarsus made possible the option of a third vector of weight transfer which could pass through the astragalus to the cuboid and digits IV-V. The primitive tarsal configuration made possible a more or less equal distribution of weight transfer to each of the digits. Artiodactyls exploited the non-serial tarsus in development of a paraxonic foot with digits III-IV becoming the main supporting digits (Fig. 1B). In the development of a mesaxonic foot perissodactyls have reduced the astragalo-cuboid contact such that the tarsus is functionally serial⁶ (Fig. 1C). Cursorial specialisation in large placental herbivores are thus built upon the primitive placental foot structure. Placental carnivores have essentially retained the primitive tarsal arrangement with modifications being most evident in the reduction of lateral digits.

The first marsupials were probably arboreal⁶⁻⁸ and most likely had a hind foot structure similar to the living South American didelphid *Marmosa*⁹. The primary structure of the marsupial pes is adapted toward prehension, and its further specialisations reflect this derivation. Primitively the marsupial pes had a moderately large opposable hallux and digits II-V were of subequal size and length. The primitive marsupial tarsal arrangement has not yet been established although a non-serial tarsus seems most probable. The vector of weight transfer from the tibia to the digits was probably similar to that of primitive placentals.

Two major groups of pedis occur in Australian marsupials¹⁰. In the family Dasyuridae (marsupial carnivores) there is an elongation of the pes and parallel arrangement of the digits as well as recession of the hallux (Fig. 1D). The ancestral forms of this family seem to have abandoned prehensile modifications for semi-cursorial ones, the pes having been made servicable for rapid progression either in the trees or on the ground. Their terrestrial ancestors have substituted digitigrade for plantigrade progression⁹. Dasyurids typically have digits II-V of subequal size although there is a tendency for digits III-IV to be slightly larger. The vectors of weight transfer are thus similar to

those in placental carnivores. In addition, the primitive dasyurid pes has a pre-adaptive potential for evolution of major cursorial specialisations similar to those seen in placental ungulates; a potential which, however, has never been exploited.

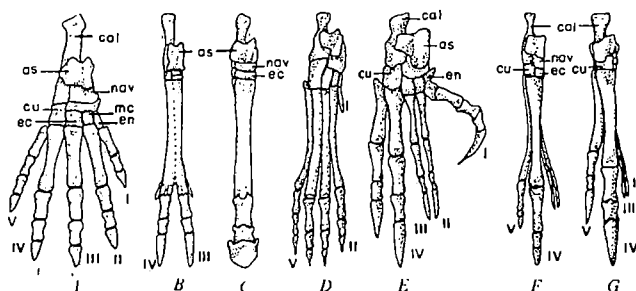


FIG. 1 Mammalian pes (right). A, Hypothetical placental ancestor; B, artiodactyl; C, perissodactyl (*Equus*); D, dasyurid (*Dasyurus*); E, phalangerid (*Trichosurus*); F, peramelid (*Macrotis*); G, macropodid (*Macropus*). A, B, C, F and G after ref. 15. Abbreviations: as, astragalus; cal, calcaneum; cu, cuboid; ec, ectocuneiform; en, entocuneiform; mc, mesocuneiform; nav, navicular; digits I, II, III, IV, V.

The second line of Australian marsupial hind foot specialisation occurs in bandicoots (family Peramelidae), diprotodonts (family Phalangeridae and its derivatives) and in the common ancestor of these two groups. Digits II-III are reduced in size and bound together in the whole of their length by a skin sheath with the exception of their end joints and claws. This syndactylous condition is deemed to be a further arboreal adaptation^{7,8} and probably arose only once in the Australian marsupials⁹⁻¹⁵, although a diphyletic origin has been advocated^{16,17}.

In phalangerids (Fig. 1E), their immediate ancestors and the immediate ancestors of peramelids, digit IV is the major support digit of the hind foot, followed by digit V, then the hallux; digits II-III are very small and have ceased to play any part in walking, running or climbing and thus do not share in the more obvious functions that are associated with toes. The syndactylous digits have become a secondarily specialised toilet implement¹³. The major vector of weight transfer is through the astragalus-calcaneum-cuboid-digit IV, although part of the body weight is also carried through the astragalus-navicular-digits I-III vector. The common phalangerid-peramelid stock had a polyprotodont dentition¹¹.

Shortly after the acquisition of syndactyly, peramelids split off as a separate lineage from the syndactylous stock¹⁰. Peramelids retained a polyprotodont dentition⁹. Some peramelids progress by quadrupedal saltation (the spring) (for example, *Isopodon*) while others (for example, *Choeropus*) have become functionally monodactylous quadrupeds (Fig. 1F). In all peramelids digit IV is the main support digit of the hind foot, a character inherited from their arboreal, syndactylous, ancestors. Peramelids have deviated from the phalangerid tarsal configuration in rearrangement of the cuboid, ectocuneiform and metatarsal IV such that the ectocuneiform has been incorporated into partial support of metatarsal IV. The tarsal configuration in peramelids allows the greater part of the body weight of the animal to pass directly from the astragalus to the distal tarsals bypassing to a great extent the calcaneum¹⁵. As a consequence the syndactylous pes of peramelids is, in part, functionally convergent with ungulates. These particular tarsal modifications, found only in the Peramelidae, have allowed a small syndactylous marsupial to become a rather specialised cursorial quadruped. This tarsal configuration would, however, be impractical for body weight transmission in a large animal. Such changes in the peramelid tarsal configuration have

been influenced by the antecedent development of syndactyly¹⁸. Peramelids and macropodids represent two independent terrestrial adaptive radiations from arboreal ancestors; similarities in foot structure are thus the result of convergence⁹.

Macropodids evolved from phalangerids after the acquisition of a diprotodont dentition⁹. The potential for cursorial specialisations in macropodids was set by the preadaptive plasticity of the phalangerid foot structure for terrestrial locomotion. As digit IV was the major supporting digit in the phalangerid pes, cursorial specialisations would have to be built around this digit in macropodids (Fig. 1G). Evolution of weight transfer vectors in macropodids incorporating digit III was restricted by the presence of syndactyly of digits II-III in their phalangerid ancestors. This restriction excluded the possibility of evolution of efficient quadrupedal cursorial specialisations similar to those occurring in placental ungulates where digit III is partly (artiodactyls) or wholly (perissodactyls) incorporated as a major support digit. Utilisation of digits III-IV together in a support function could not have evolved in macropodids¹⁴. In ungulates a vector of weight transfer is incorporated which passes weight from the astragalus to the anterior tarsals and bypasses the calcaneum⁵. Macropodids have, however, exploited the vector through the astragalus-calcaneum-cuboid-digit IV and deviated from the phalangerid tarsal arrangement in the relationship between the astragalus, navicular, cuboid, and calcaneum; in macropodids the astragalo-calcaneum and cuboid-calcaneum contact are greatly enlarged while the astragalo-navicular contact is reduced. In macropodids there is no ectocuneiform-metatarsal IV contact. This is in contrast to peramelids. Incorporation of the calcaneum into the primary weight transfer vector is a common feature among ricochet mammals^{19,20}, while in digitigrade and unguligrade mammals there has been a progressive evolution to exclude this bone from the role of supporting body weight²¹. The macropodid tarsal structure allows effective ricochet function by transfer of weight through the calcaneum. The fact that their phalangerid ancestors had already developed syndactyly and the use of digit IV as their main support digit thus predetermined the type of cursorial specialisation possible in the macropodid hind limb.

These observations provide an explanation for Howell's statement²² that "In the case of the . . . kangaroo, it is doubtful whether it is sufficiently effective at locomotion to justify the evolutionary effort put into it . . . A kangaroo is an amazingly specialised animal, but its method of traveling by saltation was hardly begun for the purpose of ultimate speed. Rather has it built speed into the locomotory pattern that was already established, probably for some other purpose".

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Banded mongoose males guard young

In most mammals care of the young is the prerogative of the female. The male, if present at all, contributes little. In the banded mongoose (*Mungos mungo*), however, males play an important role in guarding young at the den while the lactating females forage.

The banded mongoose is a diurnal social carnivore occurring in savanna habitats throughout much of Africa south of the Sahara. In Rwenzori National Park, Uganda, where I studied it for 2 yr, group (pack) size varied from approximately 5 to 40 animals. Reproduction within a pack was synchronised; several females typically came into oestrus at about the same time and subsequently produced their litters within a few days of each other. The young suckled indiscriminately from any lactating female and were groomed, played with, and transported by all pack members. They first began to leave the den for short afternoon foraging expeditions when 3 to 4 weeks old and at 5 weeks began to regularly accompany the adults in the morning. Until this age one or more pack members typically stayed at the den with the young when the others left for the morning's foraging. The pack usually returned after 2 to 4 h and for the remainder of the day the mongooses foraged intermittently in small groups. Occasionally however, a pack remained away from the den for an entire day.

To determine the sexes and identities of mongooses stay-

data for the 32 d in which marked animals stayed with the young. Twenty individuals were recorded guarding a total of 48 times. Adult males stayed with the young most frequently accounting for 73% of the total guarding records; 85% of the 20 mongooses which stayed alone with the young were adult males. The 9 marked lactating females were never recorded to stay with the young in the morning. Non-lactating females and animals less than 1 yr old occasionally guarded, but the latter did not remain alone with the young. Although the sample is small, it indicates that certain individuals show a greater tendency to stay with the young than others. In the E pack a male and non-lactating female accounted for 12 of the 17 guarding observations and two males accounted for 9 of the 11 observations in the F pack. Guarding was more equitably distributed in the I pack and 11 of the 17 pack members stayed with the young on at least one occasion.

The adaptive significance of guarding in the banded mongoose seems clear. Survival of young in the den is enhanced by the presence of an adult capable of protecting them from snakes and other ground predators while the lactating females are left free to forage. Future field studies of other species of social mongooses may reveal that male guarding of the young is characteristic of this group. My observations on *Suricata suricatta* indicate that one or more adults including males typically stay with young at the den and Rasa (personal communication) reports that this also occurs in captive *Helogale undulata*.

Male guarding in the social mongooses has few parallels in other mammals. Male marmosets of the genera *Saguinus*, *Cebuella*, and *Callithrix* are reported to transport the young from the time they are born transferring them to the female only for nursing¹. Among social carnivores, wild dogs (*Lycaon pictus*) usually leave a guard at the den with the young when the pack hunts and this role is shared in turn by females and particular males²; in most cases, however, it is the lactating female which guards although one or more males may accompany her³.

TABLE 1 Guarding of young in three banded mongoose packs

	Males	Adults		Unsexed	Juveniles and subadults (< 1 yr)	Total
		Lactating females	Non-lactating females			
Pack composition:						
E pack	8	4	2	1		15
F pack	13	3	13			29
I pack	8	3	3		3	17
Total	29	10	18	1	3	61
No. marked	21	9	13		3	46
No. marked individuals which guarded	13	0	5		2	20
No. times marked animals acted as guards	35	0	10		3	48
No. times marked animals guarded alone	17	0	3		0	20

ing with the young in the morning three packs were live-trapped and individuals ear clipped and marked in distinctive patterns with Nyanzol fur dye. All mongooses in one pack were marked but it was not possible to trap all members of the other two packs. Mongoose packs ordinarily change dens every few days. When the den site of a marked breeding pack was known, I observed and recorded the total number of mongooses leaving each morning and the identities of those marked. Any marked animals staying in the den could then be determined by the process of elimination. On some occasions a guard emerged with the pack and then returned to the young when the others left. Usually only one mongoose stayed at the den (77% of 57 observations) but up to 6 guards were recorded. On three occasions all pack members went off leaving the young alone.

Table 1 gives the pack compositions and the guarding

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book reviews

Piaget on memory

Memory and Intelligence. By Jean Piaget and Bärbel Inhelder, in collaboration with Hermine Sinclair-de Zwart. Pp. xii+414. (Routledge and Kegan Paul: London, September 1973.) £5.25.

WITH the appearance of an English translation of this book there is now available to the English speaking world a full circle of Genevan writings that focus on particular topics of cognitive functioning.

In the introductory section of the book Piaget and his co-authors link up the words "memory" and "conservation". Memory is seen as an organism's capacity to conserve something over time; but is all conservation identical with what is called memory? Conservation could include here the entire genetic past inherited by an individual, as well as the modifications he acquires during his lifetime, many of which are conserved more or less indefinitely. Piaget is willing to speak of "memory in the biologist's sense" with an extremely broad meaning for memory which includes both physiological (acquired somatic responses) and behavioural modifications.

"Memory in the wide sense" includes conservation of behavioural habits together with recall of memory images and acts of recognition. Here, however, Piaget follows the logic of his own structural theory and makes two crucial points. First, recognition, if it means the recognition of perceptual signals, is an integral part of all perceptual or behavioural habits and must be clearly differentiated from a higher-level capacity to conserve and recall an image. Second, if conservation of habits (such as eye-hand coordination or spatial know-how) is ascribed to memory, then all further action-knowledge (and for Piaget knowledge at all levels is primarily action or 'operative' knowledge) must also be included in this memory. But an operative scheme, whether on a practical (sensori-motor) or theoretical (operator) level, is not something a person remembers but something he has or is. Thus a person with the operative scheme of, say, classification does not have to remember class inclusion but simply knows that—given that London is in England—there are more people in England than in London. This kind of knowledge (or understanding) is conceptualised as being based on a hierarchy of operative schemes (Piaget calls it "schematism"). These schemes are general instruments

of assimilation of incoming data and at the same time they are accommodated when applied to particular data.

Consequently the conservation of general assimilatory schemes that are integral and permanent belongings of the person must be distinguished from the conservation of particular accommodations that took place at a certain time during the person's past. The first kind of conservation is based on internal self-regulatory mechanisms that assure the scheme's permanent functioning, whereas only the second kind of conservation can be called "memory in the strict sense". Piaget's aim in this book is to investigate the relation between the first and second kind of conservation. This aim is implied in the book's title: *Memory and Intelligence*, where memory is taken in the strict sense and intelligence means the totality of schemes available to the child. These schemes develop in the course of the child's life experiences and the resulting changes from sensori-motor to pre-operator and finally to operator stages are reflected not merely in the child's operative structure of intelligence but also in his memory. For it is hypothesised that this operative structure is essential in the structuring and restructuring of input to be retained in memory.

Parallel with the distinction just outlined goes the contrast between the operative aspect of knowing which emphasises the actively transforming and assimilating character of knowledge and the figurative aspect which emphasises the accommodation pole of knowledge, namely the relatively static character of a particular sensory configuration inherent in any concrete object or event. A related distinction—important to a discussion of memory—is between the signifying and signified (or meaning) aspect of a sign. The various distinctions can be related to this topic by stating that memory in the strict sense emphasises the figurative aspect of knowing, that recognition is basically an undifferentiated sensori-motor signal reaction, while the memory response of reconstruction and recall is a differentiated signifying symbol. A memory image is sometimes called a schema—a simplified figurative model that serves as a mnemonic device—and must not be confused with the operative scheme on which the meaning aspect of a mental image is based. None of the above distinctions must be taken in an absolute or isolated sense: they are interrelated

aspects of a person's cognitive functioning.

Within this developmental and epistemological framework Piaget and his co-authors describe their programmatic research on memory. Twenty chapters cover investigations dealing with memory for additive logical structures, multiplicative logical structures, causal structures and spatial structures. For example, there is the report in chapter 4 on the remembrance of conflicting numerical and spatial correspondences. This conflict is brought about by the display of a straight line of four matches and below it, aligned from the left, another W-shaped line of four matches. Therefore, the child saw two lines which had the same number of identical sticks but differed in left-to-right extension. After a week and again after 6 months children were required to make a memory drawing. The responses of the children were classified into five types: 1, elimination of one line; 2, two lines of equal length, one straight, the other zig-zag, of undetermined number; 3, two lines of four matches with an attempt to equalise the boundaries; 4, two lines of an arbitrary number with the straight line correctly projecting; 5, correct in number of matches and length. Types 2 to 4 indicate different ways of solving the conflict between 'same number' and 'different length'. The children's memory solutions were clearly related to developmental age; types 2 to 4 were observed in many of the younger children after a week, but only in children from 7 years on after 6 months. The authors conclude that memory after 6 months, even more than more recent recall, is subordinate to the availability of operative schemes which in the present case meant co-ordination of apparently conflicting numerical (same) and spatial (different) correspondences.

English-speaking psychologists will probably find many places where the reporting is too global and qualitative or where the table of results or the criteria for scoring are not unequivocally defined. But these investigations can and should be critically scrutinised, evaluated, replicated and modified by the scientific community. Certainly lack of methodological finesse need not blind the reader to the substantive questions on memory with which these investigations are concerned. Although Piaget's style is far from being an example of clarity, it has an internal consistency which allows the motivated reader to recognise the un-

derlying thinking. But when a professional translator, apparently without an adequate understanding of Piaget's theory, goes to work and renders the author's cumbersome French into well formed English sentences, the result, as seen in this book, can be truly disastrous. The very first pages are so full of translation errors that comprehension of Piaget's position on the basis of this introduction is out of the question.

For example, the translator does not stick to the same words in translating the original words: memory, remembrance, recognition, recall, which are definitely not interchangeable. 'Imprinting' is a technical term used by the translator where 'registration' would have been more appropriate. The French 'ou' can mean an equivalence (remembrance = conservation) or a disjunctive alternative (remembrance of schemes or of data): the translation does not clarify this ambiguity. In page 3 paragraph 3, "Habit . . . concerns that part of the memory we call recognition", is the translation given for Piaget's "habitat . . . includes recognitory memory". Where Piaget carefully distinguishes operational (in contrast to pre-operational) and operative (in contrast to figurative) the translation generally renders both words as operational; similarly, Piaget's distinction between an operative scheme and a figurative schema is largely confused.

After the introduction the reporting of the investigation is less theoretical and the translation is no longer replete with serious and confusing errors. Piaget's writings are certainly a problem, admitted by him and all, quite apart from translation. I hate to think of anything like an 'official' translation, yet this translation is not only inadequate but totally confusing as regards a potential comprehension of Piaget's psychological theory. With this proviso I recommend to all persons interested in Piaget's theory this important addition to his work on cognitive functioning and its development.

H. FURTH

Filling a nuclear gap

The Fundamental Particles. By B. H. Bransen, D. Evans and J. V. Major. Pp. vii+284. (Van Nostrand Reinhold: London and New York, September 1973.) £10.50.

THE continuing expansion of nuclear physics research and the increasing adoption of short unit lecture courses in physics departments has led to the splitting of topics within nuclear physics. This is undoubtedly good practice and the physics of fundamental particles is an obvious unit choice. This book, aimed at the undergraduate student, is well written and presented and should

serve its purpose as an introductory text. It contains a series of useful problems at the end of each chapter and is supplemented by some mathematical appendices on such topics as angular momentum, transition rates and the Weyl and Dirac equations.

After an introductory chapter, the authors concentrate for two chapters on the experimental methods of accelerating, transporting and detecting particles and measuring their masses and lifetimes. The treatment is selective but detailed. Then follow chapters on the conservation laws, symmetry principles and intrinsic quantum numbers. The pion-nucleon interaction is treated in detail using partial wave analysis. This chapter is very well put together. A chapter on mesons is followed by a discussion on the classification of particles including the quark model and the Regge classification. A description of high energy collisions follows. The authors conclude their book with chapters on electromagnetic properties and weak interactions.

The coverage is thus very extensive but the interest of the reader is well maintained throughout. The mathematics assumes some acquaintance with quantum mechanics. I find the book exceedingly readable. The introduction to each chapter is commendable. There is almost a complete absence of references in the text but a brief bibliography leads the reader onward.

This book adequately fills a gap between the simple and more advanced treatises on the subject, though its price is high.

P. R. BLAKE.

Astronomy updated

Astrophysical Quantities. By C. W. Allen. Third Edition. Pp. x+310. (Athlone), University of London. London. Distributed by Tiptree, Essex, November 1973.) £6.25.

THE eagerly awaited third edition of Professor Allen's invaluable reference book proves to be slightly disappointing. As far as updating the previous edition is concerned, Allen has done a good job, but the sections devoted to topics which have grown up since the early 1960s are less satisfactory. The "entirely new sections" include plasmas, solar wind, solar XUV, pulsars, cosmic X-rays, quasars and Seyfert galaxies. But the pulsars section consists merely of half a page defining the variables relevant to the study of these objects and a table giving the parameters of only thirteen pulsars; even three years ago a much more useful compilation of pulsar data were provided by Maran and Modali (*Earth and Extraterrestrial Sciences*, 1, 147; 1970), a source to which Allen does not even refer. It is a similar tale with

the other new sections.

The virtue of previous editions has been that they covered just about everything, removing the need to hoard articles such as that of Maran and Modali. But it is a remarkable achievement for one man to have produced the original version of *Astrophysical Quantities*, let alone ensured that the latest edition is still invaluable almost 20 years after the original appeared.

In his preface, Professor Allen suggests that the time to begin work on the fourth edition is now and asks for offers of help with its presentation. I hope that some dedicated practitioner of the new astronomy will indeed be willing to collaborate with Professor Allen on the daunting task ahead.

JOHN GRIBBIN

How behaviour develops

Behavioral Embryology. Edited by G. Gottlieb. Pp. xix+369. (Studies in the Development of Behavior and the Nervous System 1.) (Academic: New York and London, October 1973.) \$22.50.

THE first of the four sections in this book is an introduction to behavioural embryology, by G. Gottlieb. Then there are sections on embryonic motility and its neural correlates (Hamburger; Provine; Foelix and Oppenheim; Berrill); hatching: hormonal physiological and behavioural aspects (Oppenheim; Corner, Bakhuis and van Wingerden); and sensory processes: embryonic behaviour in birds (Vince; Impeken and Gold).

The subject is obviously of the first importance; few would quarrel with the idea that one of the best ways of approaching an understanding of adult form and function is to study their development. It is a pity that the misleading name 'behavioral embryology' has been adopted for what should be the embryology (or ontogeny) of behaviour.

The first two volumes of this series are intended to present a "detailed explication of the major 'philosophical', theoretical and empirical issues" involved. Since Gottlieb's introductory essay is presumably partly an attempt to explicate the philosophical, theoretical and empirical issues, it is somewhat disconcerting for the reader to come across an apparent confusion (pages 31, 32) between neural specificities and neural connections, which are not at all the same thing. Dr Gottlieb is in good company here, since this is a fairly widespread confusion. It does suggest, however, a certain laxity of approach; and this impression is not helped by a passing reference (page 25) to retinal cells connecting ("in a general way") to cells in the visual cortex.

An almost mystical element is introduced in some of the arguments in Hamburger's paper on the anatomical and physiological basis of embryonic motility in birds and mammals. Hamburger draws attention to the incongruity between neurogenesis and overt motility in amniote embryos. The unorganised movements of the 4-17-d-old chick do not, apparently, reflect the neurogenetic events going on, so to speak, below the surface. From these observations Hamburger claims that it is not valid to generalise that neurogenesis fully explains behaviour. And he makes the remarkable statement that "even the most detailed knowledge of neural organisation, including all significant synapses, in chick or rat embryos at a given stage would permit no prediction of the actual (type 1) movements performed at this stage". This viewpoint must command respect; but it surely does so as a statement of faith, of scientific position, rather than as a rigorous argument from observed data. It cannot be the latter, of course, since too many of the premises are hidden: as Hamburger points out, the observations on movement are crude and the information available on structure and ultrastructure is deplorably inadequate. After further arguments involving the results of transplanting parts of the embryonic spinal cord, Hamburger comes to the conclusion, with which I agree, that the conceptual dichotomy of coordinated against uncoordinated movements is perhaps too rigid and that such dichotomous thinking should give way to a more flexible scheme.

This book is interesting and contains much relevant information. Even arguments with which individual readers may disagree are worthwhile if they stimulate thought on this subject, where thought is badly needed.

R. M. GAZE

Computed structures

Molecular Structures and Dimensions, Vol. A1: Interatomic Distances 1960-65; Organic and Organometallic Crystal Structures. Edited by Olga Kennard, D. G. Watson, F. H. Allen, N. W. Isaacs, W. D. S. Motherwell R. C. Pettersen, and W. G. Town. (N.V.A. Oosthoek's Uitgevers Mij: Utrecht; distributed by Crystallographic Data Centre, Cambridge and Polycrystal Book Service, Pittsburgh, 1972.) n.p.

To discover the structures of molecules has been one of the main aims of chemistry since the science began. To discover the dimensions of these molecules only became possible with the advent of X-ray crystallography and various spectroscopic methods. By 1960 a very

large amount of information had been accumulated on interatomic distances and the configurations of molecules and ions. L. E. Sutton was a pioneer in the compilation of all this data, a task continued by the Chemical Society in its Special Publications.

About 1960, however, a profound change began to take place in the methods of X-ray crystallography. Fast computers and automatic diffractometers became available. The collection and analysis of X-ray measurements were vastly speeded up, and the accuracy of structure determinations greatly increased. About this time it began to be realised that the structure of an unknown molecule like a natural product could be determined more quickly and with greater certainty by the methods of X-ray crystallography than by the classical methods of organic chemistry. All this has led to a spectacular increase in the number of structure determinations from the early 1960s onwards. Up to 1972 about 8,500 organic and organometallic structures had been published, and each structure usually contains a very large amount of accurate numerical data. This output is now far beyond the capacity of the ordinary textbook to handle. But the computers that created the problem have now come to the rescue.

The Crystallographic Data Centre in Cambridge, with the sponsorship of the Office for Scientific and Technical Information have a computerised data file which contains all this information and to which about 1,500 new entries are added annually. Annual bibliographic volumes are published. The present volume is the first to give all the numerical data for organic and organometallic structures, and covers the years 1960-65. Although it modestly claims to present interatomic distances, as a continuation of Sutton's work, it does in fact cover much more than this. In addition to the chemical structural formula each structure is illustrated by a beautifully drawn stereoscopic pair (viewer provided) which has been computer generated from the published atomic coordinates. The numerical data give bond lengths, bond angles and torsion angles. These are all recomputed from the published coordinates and discrepancies noted. The torsion angles, which provide vital information concerning molecular conformation, were seldom given in papers published between 1960 and 1965 and so this volume contains a vast mine of new information. Another valuable feature is the inclusion of the *R*-factor for each structure, giving an indication of its reliability. It is interesting to note that for the 791 full three-dimensional structures listed, the *R* values range from 0.02 to 0.29, with a mean

value of about 0.10. A value of about 0.06 indicates a very accurate analysis, while a value greater than about 0.15 indicates the need for further refinement in most cases, unless the structure is extremely complex with some disorder.

The layout of the whole volume is excellent, but unfortunately it is not easy to read all the atom numbers on a few of the more complex diagrams, such as for vitamin B₁₂ and epilimonol iodoacetate. Those whose eyes do not adapt easily to stereo vision might have preferred a single larger diagram. But on the whole this is a magnificent production and Olga Kennard and her co-editors must be congratulated on their achievement. I hope and trust this heroic task will be continued with the future volumes that are contemplated. All scientists interested in molecular structure are greatly in their debt.

J. MONTEATH ROBERTSON

How to be a petrologist

Experimental Petrology: Basic Principles and Techniques. By Alan Edgar. Pp. xi + 217. (Clarendon: Oxford; Oxford University: London, October 1973) £5.75.

Books written for research workers are, in overwhelming majority, about what to think and do; they represent an attempt on behalf of the author to fashion the way his readers will approach their research problems. In contrast, this book sets out to describe how to do something: in this case, experimental petrology. It is unashamedly a recipe book destined to be fingerprinted with molykote and bleached with spots of dilute nitric acid.

In fulfilling this aim, the author has described clearly and accurately the anatomy and function of the sort of apparatus a new researcher might expect to find in any established laboratory of experimental petrology. This book will answer in advance many of the questions such a person would need to ask during their first months in the field. But the aims seem to go deeper than this as there is a quantity of detailed information about the essentially engineering side of the subject which would only be of concern to a person setting up a laboratory from scratch. I doubt whether it is realistic to hope that any book could include enough of this sort of detail to be fool-proof but at least the present work gives a reasonable guide to the literature and compares different techniques (for example starting materials) in a sensible and unbiased manner.

The level of theoretical background given is uneven. Buffers for controlling

the chemical potential of volatile species are dealt with in some detail (although I was surprised to find no reference to the work either of Burnham, Holloway and Davis or of Presnall on the PVT of water and hydrogen respectively). On the other hand, the treatment of one, two, and three component systems in chapter 2 is very cryptic; for example, the free energy of binary solutions receives less than one page and will surely convince most non-initiate readers that there is a large element of magic in thermodynamics.

But this book's great merit is that it does what it sets out to do and at £5.75 represents a worthwhile investment to anyone about to embark on research in the field of experimental petrology.

S. W. RICHARDSON

Life on Moon and Mars

Origins of Life: Planetary Astronomy. Edited by Lynn Margulis. (Proceedings of the 3rd Interdisciplinary Communications Program Conference.) Pp. xi+268. (Springer-Verlag: Berlin and New York, 1973.) DM46.70; \$16.50.

THIS is a verbatim report of the third of four conferences on the origins of life. The style of the volume follows that of its predecessors (see *Nature*, 235, 404; 1972; and 239, 175; 1972). The publisher, however, has changed, and the price has come down by over 40%. The conference, too, was organised differently. On this occasion the purpose was to explore the effect of space exploration on ideas about the origins of life. For the purpose, eight planetary astronomers, who were personally involved in space programmes then current, were assembled to meet ten practitioners interested in the origins of life (three exobiologists, two microbiologists, four chemical prebiologists and one palaeontologist). A fair proportion of the biologists had attended at least one of the previous conferences, but most of the astronomers were there for the first time. The conference was held in February 1970.

There was not the same 'free for all' that characterised the earlier conferences. On the contrary, there was a rigid division into two halves, the first devoted to the Moon, the second to Mars. In each division, certain of the astronomers were asked to discourse on aspects of their topic that they thought might interest the biologists. The biologists were encouraged to interrupt, and to ask questions. During the first division, Shoemaker, Wasserman, Kaplan and Singer discoursed on the Moon; then Oró went into considerable detail on the chemical search for carbon compounds in lunar material, and Young went into the methods

adopted in the search for microbiological activity. In the second half, Murray, Owen and Leovy spoke about Mars. Some biological interest was aroused in the part that nitrogen might or might not play in life on the planet.

But the book, if not the conference, seems to have failed in its objectives. The timing was unfortunate. Knowledge of Mars has changed dramatically with the new data collected by Mariner 9 in the following year, and the review of the Moon (which, in any case, is of less biological interest than Mars) was based on data emanating from nothing later than Apollo 11. There has been a three-year delay in publication, and the field is just moving too fast for such a leisurely programme. The conference lacked the enthusiasm of its predecessors. The book is not only out of date; it is dull. Better accounts of both the astronomy and geology can be found elsewhere. It is especially to be regretted that no attempt was made to reproduce the illustrations of lunar and Martian topography which, from all accounts, formed a major feature of the conference.

P. C. SYLVESTER-BRADLEY

Well digested Protozoa

The Biology of Protozoa. By M. Sleight. Pp. viii+315 (Arnold: London, October 1973.) £7.50 boards; £3.75 paper.

RECENT spectacular increases in knowledge of the genetics, biochemistry and ultrastructure of the Protozoa have greatly increased the difficulty of writing a satisfactory book of modest size on this group. Dr Sleight has attempted to produce in 300 pages, at about degree level, a general biology of the Protozoa, for the most part successfully.

After a brief introduction on evolutionary relationships and cellularity, three chapters are concerned with structure, metabolism and reproduction. Of the remaining seven chapters, six are devoted to taxonomic groups. It seems to me regrettable that the general treatment with which the book begins (and ends: chapter 11, "Ecology of Protozoa") was not carried through completely. But repetition, which is liable to occur in combining a systematic with a subject approach, is largely avoided by numerous cross references. Complex ascriptions in the text are minimised by the numerical reference system; hence the presentation is one of digested rather than of quoted information. Occasionally one could wish for more references to the sources of information, as in Table 5.1 of "Features of Various Flagellate Groups".

The illustrations are of diverse kinds, light and electron micrographs, draw-

ings and diagrams and are generally fresh and apposite although some of the drawings of whole animals made them look, to me, more solid and opaque than protozoa usually do in the microscope.

This is altogether a valuable and timely book of the right size for use in undergraduate courses.

G. CHAPMAN

Mediterranean collection

The Mediterranean Sea: A Natural Sedimentation Laboratory. By D. J. Stanley. Pp. xvi+765. (Dowden, Hutchinson and Ross: Pennsylvania; Wiley: Chichester, September 1973.) £22.50.

THIS volume is a collection of forty-seven papers brought together out of the feeling that the Mediterranean Sea is an ideal environment for the study of marine sedimentation, and in the belief that sedimentological work in this area, cutting across linguistic and national boundaries, should be summarised in order to speed future investigations. The papers are divided between twelve sections touching on many different aspects of the Mediterranean, its bathymetry and hydrography, the geological setting up to and including the Quaternary, clastic and non-clastic sedimentation on shelves and in coastal areas, deep-water sedimentation, and geochemistry. There is a paper on pollution of the Mediterranean and another sketching which topics should be given priority in research in the next decade.

Although a commendably high editorial standard is apparent, the content and relevance of the papers are uneven and not wholly representative of recent work in the Mediterranean. Some papers are regional or subregional in scope, whereas many deal with what could be purely local problems, and a few simply use the Mediterranean as the setting for the study of a general topic. Among these personal original endeavours, I sorely missed attempts at regional syntheses specific to the Mediterranean, which would aim to show in detail what was known and what remained to be studied. It is easy from this book to discover fairly completely who is doing what and how—it is excellent as a compilation transcending national differences in scientific outlook—but I am less confident that a critical light has been focussed on the major research problems of the Mediterranean and its hinterlands. A different blend of original papers and critical reviews might perhaps have led to a book greater than the sum of its parts.

J. R. L. ALLEN

matters arising

Evolution of the Tasman Sea

SIR,—Hayes and Ringis¹ have presented an interesting model for the evolution of the Tasman Sea. I wish to call attention, however, to what I feel is an inappropriate reference to an earlier article by myself and Brennan². Hayes and Ringis¹ state that: "A cursory examination of widely spaced east-west aeromagnetic profiles between Australia and the Lord Howe Rise led to the early but incorrect conclusion that there were no lineated magnetic anomalies within the Tasman Sea." But in discussing the possibility of lineated anomalies in Tasman Sea we stated that: "While the lack of correlated north-south anomalies cannot be proved conclusively from our data, if such correlation does exist it is not apparent."

Furthermore we said: "But the fact that the individually widely spaced magnetic profiles resemble magnetic profiles produced by the process of seafloor spreading means that the possibility that these magnetic anomalies were produced by an earlier episode of seafloor spreading cannot be excluded. This earlier period of seafloor spreading could have been complex, requiring a closely spaced survey to detect its presence." From our study we concluded that: "A more detailed survey should be conducted to resolve these two contradictory ideas (that is, lineated as compared with nonlineated anomalies) concerning the source of the anomalous magnetic pattern formed in the Tasman Sea."

Unfortunately, some confusion may result from the title given to our letter

on the index page of the issue in which it appeared. It was referred to as: "No seafloor spreading in the Tasman Sea," perhaps an unfortunate summary in view of the above.

My present comment does not, of course, effect the substance of the Hayes and Ringis paper but merely seeks to clarify the interpretation of an earlier reference.

Yours faithfully,
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Washington DC 20373
USA

¹ Hayes, D. E., and Ringis, J., *Nature*, **243**, 454 (1973).

² Taylor, P. T., and Brennan, J. A., *Nature*, **224**, 1100 (1969).

science on television

Under the BBC's bushel

John Gribbin and Fiona Selkirk

IF you ask the average scientist in the street (let alone the apocryphal man on the Clapham omnibus) what science programmes are to be found on British television he would probably reply "Well, there's 'Horizon', of course, and the Open University, and I think there was a programme about anorexia on ITV the other day, and sometimes odd bits late at night on BBC." In fact, those odd bits amount to regular broadcasts in 30 weeks of the year (excluding repeats) under the guise of further education. But the BBC in general, and *Radio Times* in particular, is so coy about this output that it is difficult to find what is on, and when, at the best of times. Recently, these programmes have suffered because of restrictions on late night television (in Britain that means after 2230) and because of the subsequent domination of our screens by election programmes; so these difficulties have been greatly enhanced. We therefore found it necessary to resort to a visit to the BBC Tele-

vision Centre, where several hours spent in a viewing room provided the material needed to comment on this almost undercover output from the BBC.

Of the forthcoming attractions "The Experimenters", provisionally scheduled to be shown on BBC1 on Mondays from April 22, will probably be of considerable interest to a scientifically knowledgeable viewer.

It is seldom possible to combine scientific research with vocational work and the choice after graduation is a difficult one to make and even more difficult to go back on if the choice transpires to have been wrong. A doctor in a mining community in South Wales has, however, managed to do just this and plans to provide some useful information about the occurrence and development of gut cancer, while still practising medicine in the community. He is the 'experimenter' in one of these programmes. The series attempts, and if this example is representative, succeeds, in looking at the people behind scientific research, their methods and motives.

This doctor tried pure research and decided he wanted to know his 'guinea pigs' as people. He took up general

practice and is starting a survey into gut cancer using his patients as the subjects.

The scene of his meeting with the local community in one of the local clubs—on Bingo night—is effective. The attentive, slightly worried faces of his audience are intriguing as he explains what he is trying to do. "Why is this survey being done here and not in London?" he was asked. With a wry smile, he explained about population stability and community spirit, features not exactly characteristic of the population of an average big city.

There are problems involved with doing this kind of research, the biggest being communication, but this doctor argued convincingly that the feedback from research improves his value as a general practitioner even if the research turns out not to be of any great significance in itself.

Even in the laboratory, however, the halcyon days of scientific breakthroughs being made by a lone researcher are gone and today scientists have to work in teams. The choice of team mates is critical or research can grind to a halt through personality differences, even though the minds behind those per-

sonalities may have the same goal.

This concept was well illustrated by the differing attitudes to the controversial results of some research by a team of three more 'experimenters' in the series.

Drs Walter Kellerman, Gordon Brooke and John Baruch are now famous as the team that might have discovered a quark, or not, as the case may be. Baruch is brashly convinced of the validity of their cosmic-ray experiments, and hustled his colleagues into publication. "Why let someone else get the credit?", he argued. Brooke seemingly regrets this haste and says that he would not have published this claim without more evidence, if he had had his way. And Kellerman, balancing these extreme views and presumably with the casting vote, decided in favour of some publicity, to encourage further work in the field (and, no doubt, to encourage further grants to the group at the University of Leeds).

The discussions between the members of the team certainly showed the human face of science (with a few small warts); and Kellerman was heretical enough to point out that the 'scientific method' is a mythical beast.

Two other programmes came from series already screened and perhaps even seen by those people who were able to find out that they were on. A look at some of the 20,000 or so tiny creatures that share the average house with its

human occupants was not for the squeamish adult but, overall, with light-hearted narration, a remarkable range of background music and some expert photographic work, it was just the stuff to give an appreciative audience rather earlier than 2325—although perhaps not just after supper.

The combination of some of the best photographic techniques (the use of light and scanning electron microscopy, time-lapse and excellent closeup camera work) makes this extremely successful visually, rivalling the best of "Horizon", "The World About Us" and others.

As for "Bellamy on Botany", Dr David Bellamy has, through the force of his own personality, ensured that his contributions to the further education output are reaching a wide audience. Bellamy has the character and personality to carry a programme transmitted live from a sewer (perhaps this will come in due course), but the programme we saw came from the clean dunes of Holland; we learned how natural reclamation of land from the sea takes place as plants establish stable dune formations.

The comparison with programmes such as "Horizon" is again favourable—especially so since, as we were told, a number of further education programmes can be produced for the cost of one "Horizon". These programmes may be labelled education, and that may put off some casual viewers, but as we saw they can be as hard hitting as any cur-

rent affairs programme, or as entertaining as any feature, as well as being educational.

And what of programmes aimed specifically at schools? One silver lining to the recent rail travel troubles in southern England has been the opportunity to view some of these, along with several thousand schoolchildren and an unknown number of housewives. On the basis of this limited opportunity, the scientific programmes for schools, especially those for 16 to 18-year-olds, are every bit as good as their late night counterparts. Indeed, many of them could be shown unchanged in the evenings, and it is rather odd that although further education programmes are sometimes repeated for schools, the reverse flow is non-existent. Here, surely, is a useful way to occupy the 22 weeks when further education programmes disappear from their usual slot.

But one thing is certainly clear. There is no need to worry about the health of science on television in Britain; it is alive and well, and living under the name of "further education". What is worrying is that these programmes receive little or no promotion, and that those responsible for screening and publicising the programmes seemingly lack the imagination of the people who produce them. Until this situation is improved, a little research into the small print of the *Radio Times* is likely to prove a very rewarding exercise.

Announcements

Erratum

In the article 'Lyoluminescent tissue equivalent radiation dosimeter' by Nadir A. Atari and Kamil V. Ettinger (*Nature*, 247, 193, 1974) there is a misprint in the title: 'dosimeter' appeared as 'desimeter'. Also paragraph 6, line 10 should read ... 'between 1 and 10⁶ rad ...' not ... 'between 1 and 60⁶ rad ...'.

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

Science Research Council—Electrical and Systems Engineering Committee. Report on Electrical Machines. Pp. iv+19. (London: Science Research Council, State House, High Holborn, 1973.) gratis. [2410]

Department of the Environment. First Report on Research and Development 1973. Pp. vi+56. (London: HMSO, 1973.) 42p net. [2410]

The Mathilda and Terence Kennedy Institute of Rheumatology. Sixth Annual Report. Pp. 67. (London: The Mathilda and Terence Kennedy Institute of Rheumatology, 1973.) [2510]

Department of Education and Science. Safety in Science Laboratories. (DES Safety Series, No. 2). Pp. iv+39. 32p. Safety in Practical Departments. (DES Safety Series, No. 3). Pp. iv+43. 32p. Safety in Physical Education. (DES Safety Series No. 4). Pp. iii+22. 21p. (London: HMSO, 1973.) [2610]

Final Report of the Anti-Locust Research Centre, 1 January 1970–31 May 1971. Pp. 72. Centre for Pest Research—Descriptive Brochure. Pp. 24. Centre for Overseas Pest Research—Report June 1971–December 1972. Pp. 148. (London: Centre for Overseas Pest Research, 1973.) [2610]

Other Countries

Scanning Electron Microscopy of Ascosporic Aspergilli. By R. Locci. (Supplemento al Vol. VIII, Serie IV, 1972 della Rivista de Patologia Vegetale.) Pp. 172. (Milano: Istituto di Patologia Vegetale, Cattedra di Micologia, Via Celoria 2, 1972.) [2210]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 72-12: Subsurface Lower Paleozoic Stratigraphy in Northern and Central Alberta. By D. C. Pugh. Pp. iii+54. \$5. Paper 72-47: Geology of Tavani Map-Area, District of Keewatin. Pp. iii+14. \$1.50. Paper 73-19: Paleomagnetic Results from the Tertiary Mount Barr and Hope Plutonic Complexes, British Columbia. Unit Correlations and Tectonic Rotation from Paleomagnetism of the Triassic Copper Mountain Intrusions, British Columbia. By D. T. A. Symons. A Ballistic Magnetometer for the Measurement of Rock Magnetic Properties. By E. J. Schwarz and T. Whillans. Pp. iv+34. \$2. Paper 73-21: Field and Laboratory Methods used by the Geological Survey of Canada in Geochemical Surveys. No. 12: Mercury in Ores, Rocks, Soils, Sediments and Water. By I. R. Jonasson, J. J. Lynch and L. J. Tripp. Pp. iii+22. \$2. Paper 73-29: A Grenville Front Magnetic Anomaly in the Megiscane Lake Area, Quebec. By B. W. Charbonneau. Pp. iii+20. \$2. (Ottawa: Information Canada, 1973.) [2210]

Lawrence Berkeley Laboratory. Research Highlights 1972. Pp. 60. (Berkeley: Lawrence Berkeley Laboratory, University of California, 1973.) [2210]

CERN—European Organization for Nuclear Research. CERN 73-9: Diagrammar. G. 't Hooft and M. Veltman. Pp. iv+114. (Geneva: CERN, 1973.) [2210]

Smithsonian Contributions to Zoology, No. 147: The Old World Stenomitridae: a Preliminary Survey of the Fauna, Notes on Relationships, and Revision of the Genus *Eriogenes* (Lepidoptera: Gelechioidea). By W. Donald Duckworth. Pp. 21. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) 40 cents. [2210]

United States Department of the Interior: Geological Survey. Professional Paper 764: Stratigraphy

of the Southern Coast Ranges near the San Andreas Fault from Cholame to Maricopa, California. By T. W. Dibblee, Jr. Pp. iv+45. 80 cents. Professional Paper 765: Geology of the Oxidized Uranium Ore Deposits of the Tordilla Hill-Deweesville Area, Karnes County, Texas; a Study of a District Before Mining. By C. M. Bunker and J. A. Mackallor. Pp. iii+37. Professional Paper 775: Petrography of Some Granitic Bodies in the Northern White Mountains, California-Nevada. By Dwight F. Crowder and Donald C. Ross. Pp. vi+27. 70 cents. (Washington, DC: Government Printing Office, 1973.) [2210]

United States Department of the Interior: Geological Survey. Professional Paper 747: Pennsylvanian Carbonates, Paleocology, and Rugose Colonial Corals, North Flank, Eastern Brooks Range, Arctic Alaska. By Augustus K. Armstrong. Pp. v+21+8 plates. (Washington, DC: Government Printing Office, 1972.) \$1. [2310]

US Department of Health, Education and Welfare. National Institutes of Health. DHEW Publication No. (NIH) 74-575: Soviet Medicine—a Bibliography of Bibliographies. (A Publication of the Geographic Health Studies Program of the John E. Fogarty International Center for Advanced Study in the Health Sciences.) Pp. vii+46. (Washington, DC: Government Printing Office, 1973.) 80 cents. [2310]

Methods for the Analysis of Human Chromosome Aberrations. Edited by K. E. Buckton and H. J. Evans. Pp. 66. (Geneva: WHO; London: HMSO, 1973.) 12 Sw. francs; £1.50; \$3.60. [2410]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 219: Lower Cretaceous Bullhead Group Between Bullmoose Mountain and Teesa River, Rocky Mountain Foothills, Northeastern British Columbia. By D. F. Stott. Pp. 228 (15 plates). \$6. Bulletin 222: Contributions to Canadian Paleontology. By D. E. Jackson, B. S. Norford, D. S. Broad, D. L. Dineley, A. E. H. Pedder, A. R. Ormiston and L. Cameron Mosher. Pp. 192. \$6. Paper 73-11: Publications on the Geology of the Arctic Islands by the Geological Survey of Canada. Compiled by R. L. Christie. Pp. 39. \$1. Paper 73-36: Studies in "Standard Samples" of Silicate Rocks and Minerals. Part 3: 1973 Extension and Revision of "Usable" Values. By Sydney Abbey. Pp. 25. \$1. (Ottawa: Information Canada, 1973.) [2510]

Smithsonian Contributions to Zoology, No. 152: Evolution of the Rails of the South Atlantic Islands (Aves: Rallidae). By Storrs L. Olson. Pp. iii+53 (11 plates). (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) 95 cents. [2510]

nature

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Outlook with Labour

MR WILSON has eventually secured what most expected him to, and the political outlook, precarious as it is, is unlikely to force him into another election before he has had time to govern for six months to a year.

It was apparent immediately Mr Wilson announced his Cabinet that this was not to be a palliative government, shaped to defuse the Liberals and other parties. Three key posts, Employment, Industry and Trade go to three left-wingers Messrs Foot, Benn and Shore—vigorous proponents of nationalisation and renegotiation of the European Treaty. Unlikely as it is that moves towards nationalisation will come in the next few months, it is almost certain that within a year this Labour Government would be moving towards public ownership in several industries.

The three appointments that will most interest British scientists are those of Mr Roy Mason to Defence, Mr Eric Varley to Energy and Mr Reg Prentice to Education and Science.

Defence isn't what it used to be. The great debates on Britain's nuclear deterrent and her world posture are now part of history and there is a deep lack of interest in defence problems. The only highly visible role of the armed forces is in Northern Ireland, the last and most insoluble of the colonial peace-keeping problems. This is an operation that many tough-minded citizens would like to see terminated 'to let the Irish sort it out for themselves', and certainly few outside military circles give military matters much thought.

This is a bad situation. The military mind left to its own devices will expand outrageously. Mr Mason is committed by the Labour Party's manifesto to prune hundreds of millions of pounds off defence spending and will meet fierce resistance. In the past two years military research and development expenditure, having declined steadily for years as a fraction of total military expenditure, suddenly started to rise again. Much is in the pipeline, such as the Multi-Role Combat Aircraft and improvements to the Polaris fleet. Mr Mason will, no doubt, squash plans for the refitting of Polaris. He might well start enquiries into the long term future of anti-submarine warfare and nuclear weapons development.

Mr Varley comes into a young ministry and has many well-wishers. Not, as one newspaper put it, because "his mining experience should be useful" as if years of handling coal gave one a special insight into energy problems shared only by petrol-pump attendants. Rather, his is a relatively unknown face and there is hope that a fresh mind, not too blunted by previous political battles, could do a good job in this demanding ministry.

The first major issue that he will have to consider is the nuclear reactor question. This is one of those de-

cisions in politics which despite all the finely calculated analyses and extensive documentation will be made on instinct. Noises before the election suggested that the instincts are rather strongly against light water reactors, and in favour of the very cautious step of more magnox reactors.

Mr Prentice is rather a surprise at Education and Science. He had been at the ministry before at a junior level, but has recently been 'shadowing' employment. However, his well-known dislike of the left-wing made that an impossible post for him. Education and Science may still only be a temporary staging post.

It is most unlikely that science will see any major changes in the year ahead other than those caused by financial constraints. Labour has no plans for altering the present relationship between research councils and the research they sponsor, so for the time being the Rothschild restructuring will presumably be allowed to proceed. Labour likewise does not intend to implement the recent recommendations for student loans and for changing the emphasis to post-experience study for PhDs.

Finally, what of some of today's symbols? Concorde, child of the white hot technological revolution of the 1960s, will no doubt fly for BOAC and Air France and few else: this is a business decision and politicians will be glad to be rid of it. Maplin, London's third airport, will have to wait. And the think tank? One suspects it will stay, and that one or two of *Nature's* readers will end up in it.

100 years ago



IN a most interesting article on the planet Mars, in your issue of *NATURE* for Feb. 19, which has just been shown to me, the Rev. T. W. Webb directs attention to the question of the colours of Mars being due to effects of contrast or not, and says—"Nor does it seem to have been noticed that no effect of contrast has been traced in the Polar snows."

Kindly permit me to inform Mr. Webb that, in a paper on Mars in the last volume of the "Monthly Notices of the Royal Astronomical Society," I expressly state that, "on May 14, 1873, the south Polar ice appeared (in an 8½-inch silvered glass reflector, by Browning) of quite a pale sky-blue colour, evidently by contrast," and I may add that this effect I noticed also on two or three subsequent occasions.

Burton-on-Trent, March 12

EDWARD B. KNOBEL

From *Nature*, 9, 247, February 12, 1874.

Scientists' dispute is symptom of a deeper problem

Dr G. J. Leigh, of the Agricultural Research Council Unit of Nitrogen Fixation at the University of Sussex, expresses his personal views about the pay dispute involving British Civil Service scientists.

THE current agitation among Civil Service scientists is stimulated by comparisons of pay scales such as those in Table 1. Scientists have been in dispute with the Civil Service Department (CSD) for some four years but I shall not detail the history of the dispute here. Its resolution currently lies with the Pay Board but is not yet in sight. It is the CSD's opinion that the question is not one of relativities and Lord Windlesham has now implied that the government is not bound to accept the findings of the Pay Board. In November 1973, at the inception of Stage III, the CSD negotiated with indecent haste a pay settlement for about 400,000 civil servants, but excluding scientists, giving increases of nearly 20%, and there is a feeling among scientists that the CSD is conducting a vendetta against them. How else to explain the situation in which an officer with 3 A-levels can qualify for the Family Income Supplement?

The current situation is, however, a reflection of conditions which have existed for many years. The government employs more scientists than any other organisation in the country yet it is far from clear whether the government—any government—has ever had a coherent policy for science and technology other than allowing developments to occur by default. Any government of a modern state requires scientists to carry out routine tasks but it has been accepted for more than fifty years, since Haldane at least, that the British government should sponsor general long term research and have available a source of informed scientific opinion. Presumably this requires a pool of scientists of the highest quality. But although an individual can define areas in which he feels the government should be active, there is little indication that a government knows how it should use its scientists or even that it really wants to.

The 1964 Labour government talked about the white heat of technology but the only long lasting product of the

conflagration would seem to be Concorde. The 1970 Conservative administration carried through the Rothschild exercise. And it cannot be denied that any group which consumes large amounts of state money has to recognise that it is accountable to the state. Scientists are perhaps more cautious and conservative than most but the manner of introduction of Rothschild principles was not calculated to allay their apprehensions. One consequence was the creation of uncertainty among a large group of scientists who felt they were being forced into a new kind of organisation of which they did not necessarily approve. Another was to enlarge the bureaucratic machinery through which research projects must filter before being acted on.

Lord Rothschild did not attempt, however, to define the areas in which applied research should be carried out. He left the selection of work area to a rather artificial market mechanism such as has operated (sometimes with conspicuous lack of success) for defence contracts. Nor did he deal with pure research, which was outside his immediate terms of reference. Consequently, research programmes must become dependent on specific pressures from industry or ministries, and for that reason are likely to become primarily short term in nature.

Nevertheless, the Rothschild mechanism does represent a way in which a government could introduce a broad coherent policy covering both pure and applied research, and encompassing problems such as cancer, fuels or resources. It also implies a scientific Civil Service which has a properly developed structure in which problems such as pay relativities, short term contracts, transfer between Civil Service classes and career development in small research units, have been fairly resolved.

The reality is rather less encouraging. For example, one hears through the grapevine that there is money 'available' for research into energy and resources problems. It would seem that this will produce some postgraduate and post-doctoral research in a few university departments rather than be applied in a broad, considered approach to national problems. The response is too little, too timorous and probably too late.

The application of the Fulton principle of unified pay and grading in the Civil Service, which would solve many of the problems outlined, has been supported by both Labour and Conservative governments. Even while it has been official government policy, the consequence of the CSD's actions has been to make the implementation below the senior grades more difficult. Ministers have expressed deep regrets about the current situation but nothing more concrete has transpired. Consequently Civil Service scientists have become much more aware of the need to organise themselves and of the need to have a strong trade union,

TABLE 1 Relative pay scales for Civil Service groups at January 1, 1974 and numbers in post on April 1, 1973

Science Group	No.	Salary range (£)	Administration Group	No.	Salary range (£)
Principal Scientific Officer	2,140	4,895-3,715	Principal	3,778	5,775-4,360
Senior Scientific Officer	3,505	3,895-2,798	Senior Executive Officer	6,403	4,542-3,756
Higher Scientific Officer	3,904	2,854-2,221	Higher Executive Officer	19,996	3,585-2,953
Scientific Officer	2,898	2,329-1,410	Executive Officer	45,670	2,782-1,819
Assistant Scientific Officer	3,800	1,729-792			2,306-1,360
Subtotal	16,247		Total	75,847	
In fringe bodies (for example United Kingdom Atomic Energy Authority research councils)	11,206				
Linked grades (for example Patent examiners, Defence College lecturers)	4,501				
Total	31,954				

Experimental Officer and Scientific Officer and Assistant Scientific Officer are not exactly comparable

whether inside or outside the Trades Union Congress.

I have tried to show that the current pay dispute is only a symptom of a much deeper problem, which is how (and possibly whether) the government should use science and scientists. Certainly scientists will have to learn to be more adaptable but they cannot be treated as operatives

who perform a service to order. It is the duty of a government, through its science policy, to create the environment in which scientists can work constructively. It is in the interests of scientists and the community in general that the current impasse be resolved and not be allowed to re-develop.

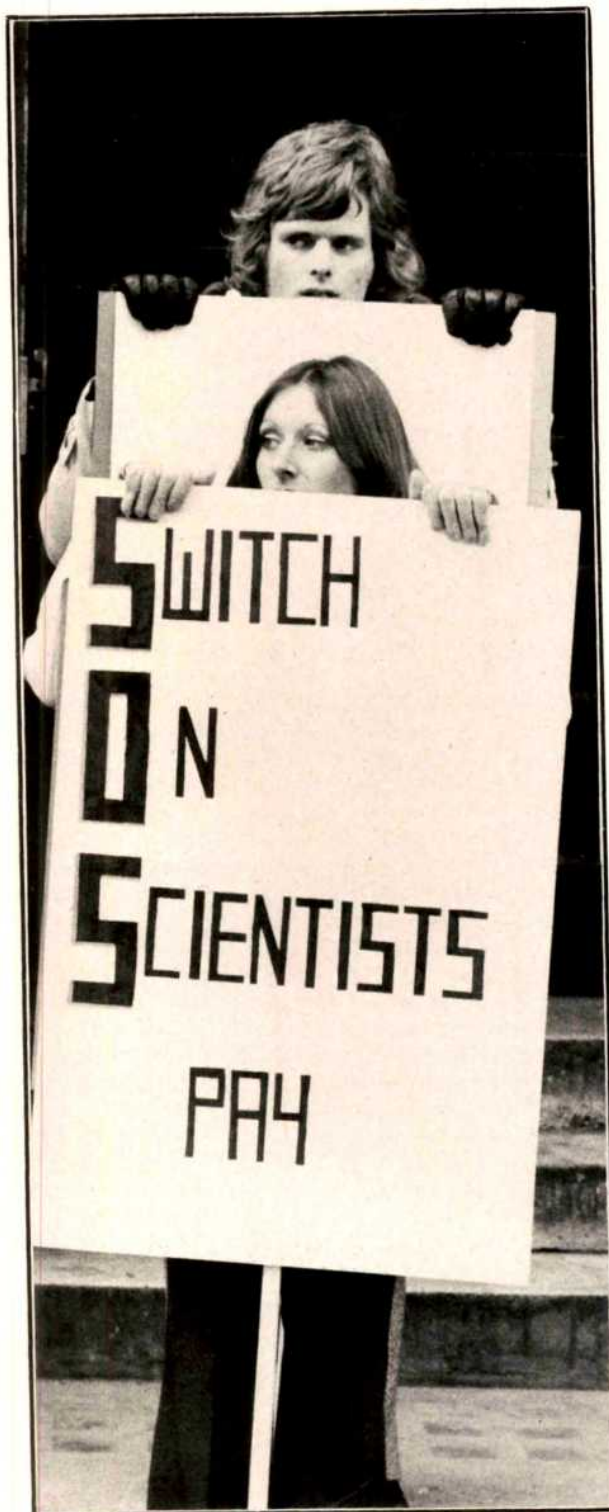
Picture by Robin Laurance

Scientists on the march

FOUR thousand government scientists and a labrador dog, led by a jazz band playing the blues, made up the first major demonstration to converge on Number Ten, Downing Street, during Mr Wilson's latest tenancy. After a mass meeting at the Central Hall, Westminster, where striking scientists signalled their militancy with well ordered applause at their general secretary's every cadence, the demonstrators bore down on the Prime Minister, and at one stage their parade filled Whitehall from one end to the other. The occasion was the first national half day strike by members of the Institution of Professional Civil Servants (IPCS), whose campaign to force a decision on their long delayed pay claim happened to coincide with a general election campaign. Indecision over the state of the parties, and their relative abilities to form a government, left the scientists with a grand scale protest planned and no minister in office to enjoy the benefit of it. So they settled for Mr Wilson, who on a bright afternoon, to the strains of the St Louis Blues, received the following note:

"You will know that civil service scientists and those employed in fringe bodies have today been on strike in connection with the completely unreasonable delay in dealing with their long outstanding pay claims. This is the first occasion on which any group of the institution's members have taken national action of this kind, and it is the clearest possible demonstration of their dissatisfaction with the present position. At a meeting of scientists employed in the London area, at which something like 2,000 were present, the following resolution was carried: this mass meeting, representing thousands of scientists employed in the Civil Service and fringe bodies in the London area, expresses its deep sense of outrage that after three years of patient endeavour the government is still procrastinating on scientists' reasonable pay demands. It stresses the absolute necessity for immediate pay increases for scientists who are unique in having suffered a fall in living standards over the past three years and who are now at least 28% behind general pay movements. It urges the government to give priority to producing a fair and satisfactory solution to this urgent problem."

The Pay Board's report on scientists' pay, the delay of which caused the IPCS to mount a campaign of industrial action, is expected under the new administration to be published rather promptly. Not only does Mr Wilson have a distinct commitment to placate angry unions, but the Pay Board, whose future may well hang in the balance, does not have much else to work on these days. The SOS placard in the pictures is a new slant on a slogan which the outgoing government coined in order to save power during the miners' strike: "Switch off something". The electorate apparently got the message.



international news

Academy reports on Vietnam herbicide damage

Colin Norman, Washington

IN the late 1960s, United States military forces dumped nearly 19 million gallons of herbicides on an area of South Vietnam equal in size to the entire state of Connecticut or Northern Ireland. According to the National Academy of Sciences, the most prestigious scientific organisation in the United States, some of the sprayed areas could bear the scars of this massive chemical assault for 100 years or more unless swift action is taken to reforest the land. The academy has also reported that interviews with people living in the highlands of South Vietnam have produced extremely consistent reports that children and animals were killed by the herbicide sprays.

Those conclusions have emerged from an intensive study conducted by an international team of scientists which worked for three years under the academy's auspices. After much delay and considerable wrangling in the academy itself, the fruits of the team's endeavours were published last week in the form of a 400-page report* prepared for the Department of Defense and the US Congress. The document provides the most extensive and authoritative survey so far of the damage caused to the Vietnamese people and their environment by military herbicide operations, but it is unlikely to quell the bitter debate that has been going on in the United States about this aspect of the Vietnam war.

For one thing, the report is already being used by both sides to bolster their cases. The Department of Defense, for example, has put out a statement which invites the reader to draw the following conclusion from the report: "some damage has resulted from the military use of herbicides in Vietnam, however, most of the allegations of massive, permanent ecological damage are unfounded". On the other hand, it has been argued that

the academy has turned up evidence of damage so extensive that it provides a compelling case for a flat ban on the use of herbicides in war.

Another reason why the report is unlikely to dampen the debate is that the investigating team has failed, for a variety of reasons, to come up with a definitive answer to the most contentious question of all—whether or not the herbicide spraying caused an increase in the number of birth defects in communities that were particularly hard hit.

Finally, the team itself failed to reach agreement on how much destruction has been wrought on Vietnam's inland forests. This aspect, in fact, held up publication of the report, caused three members of the team to disassociate themselves from the final conclusion, and is sure to lead to a long dispute.

The report, nevertheless, is an extremely valuable document, not least because it is the first authoritative account of the extent and nature of the military herbicide missions flown over South Vietnam between 1965 and 1970. In all, nearly 9% of the area of South Vietnam received at least one dose of herbicide.

Working from a military record which gave details of the time, place, type and amount of herbicide sprayed, in conjunction with vegetation maps and aerial photographs, the academy team constructed a picture of the spray operations which indicated that 10.3% of South Vietnam's inland forests, 3.2% of the cultivated land, 36.1% of the mangrove forests and 5.5% of other areas in the country were doused with a variety of herbicides at least once.

In the total of nearly 19 million gallons of herbicide distributed over South Vietnam, the majority—11.22 million gallons—was so-called 'agent orange'. This is a half-and-half mixture of the herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). Use of this agent was terminated in 1970, according to the military records, because it had been suggested that 2,4,5-T may cause birth defects, and because it was found that the stuff was contaminated with a highly toxic chemical called TCDD (2,3,7,8-tetrachlorodibenzo-para-dioxin). The other two chief agents used were 'agent white' (2,4-D and Picloram) and 'agent blue' (sodium cacodylate, and arsenic compound).

According to the military records, about 88% of the spraying was directed at forests, 9% was aimed at destroying crops in areas under the control of the National Liberation Front (NLF), and about 3% as directed at the perimeters of military bases. But those figures underestimate the area of crops destroyed for a variety of reasons. For one, spray from defoliation missions tended to drift outside the target area on to crops; in fact, the report states that "in 16 out of 18 areas studied by aerial photography, crop damage by defoliation missions was greater, and sometimes considerably so, than by crop destruction missions". For another, the Montagnards (the highlanders) conduct much of their agriculture in cleared areas of forests, and so their fields were often sprayed during attacks on the inland forests. Finally, the report suggests that in some defoliation missions, herbicide was in fact sprayed directly on crops, although the investigating team is careful to say that their suggestion "is not intended as an expression of opinion as to whether or not the official policy on herbicide operations was violated".

The damage wrought by this assault varied considerably from one area to another, but the most severe and the most persistent destruction was suffered by the mangrove forests. The report indicates that a single spray attack on a mangrove forest was sufficient to kill all the trees directly hit, and some regions of South Vietnam have been completely denuded by repeated herbicide applications.

More than a third of all the mangrove forests in South Vietnam were directly sprayed, according to the records, and the report states that "large contiguous areas were devastated, and there has been little or no recolonisation of mangrove trees in extensive areas". The reason is that some regions, such as the Rung Sat zone southeast of Saigon, were so heavily sprayed that all the vegetation has been destroyed and no seed sources are left to colonise the area. The report states that "under present conditions of use and natural regrowth, it may take well over 100 years for the mangrove area to be reforested". A massive reforestation programme, however, could probably restore the mangrove forests in two or three decades.

Although those reports are bad enough, some observers have suggested

* *The Effects of Herbicides in South Vietnam*, Part A: Available from the National Academy of Sciences, 2101 Constitution Ave NW, Washington, DC 20418. \$10.

that they may even underestimate the extent of damage to the mangrove regions. Dr Matthew Meselson, a Harvard biologist who surveyed the effects of herbicide damage for the American Association for the Advancement of Science, suggested in a telephone interview last week, for example, that uncertainties about the extent of the area sprayed, drifting outside the target area, and other factors may have caused the academy team to underestimate the sprayed area by about 30%. In any case, Dr Philip Handler, President of the academy, points out in a personal introduction to the report that unless a vigorous reforestation programme is conducted in the mangrove forests "mankind will have been guilty of a large and ugly depredation of our natural heritage".

Although the academy team and other observers are in relatively close agreement about the massive devastation suffered by the mangrove forests, the effect of herbicide spraying on inland forests is open to considerable debate. Because much of the survey was carried out while the war was going on, and because even after the cease-fire was signed a considerable amount of fighting was still taking place, the academy team had to rely chiefly on aerial photography for its analysis of the destruction of inland forests. Under those conditions, little ground work was possible. Consequently, the data are not of the best quality, and their interpretation is, to put it mildly, a difficult task.

When the report was in one of its early drafts, it became apparent that the investigating team's estimate of the destruction was considerably below that of other observers and investigators who had surveyed the damage for themselves. The academy's Report Review Panel, a committee of academy members which screens publications before they are released, refused to accept the estimates, and instructed the team to take another look at the data. It later became apparent that the extent of damage was underestimated because little account had been taken of the fact that repeated herbicide applications killed the trees to a greater extent than the team had first supposed.

Three independent photointerpreters and forestry experts were called in to help with the analysis, and the report eventually came up with the suggestion that about 1.25 million cubic metres of 'merchantable' timber had been lost from the inland forests because of herbicide spraying. The report said that although the exact figure cannot be taken as Gospel truth, it almost certainly lies between 0.5 and 2.0 million cubic metres.

Two of the independent photointerpreters reckoned that the figure is probably near 2.0 million cubic metres, while the third said that it could be an order of magnitude greater. One of the team, Professor Pham-Hoang-Bo from Saigon University, dissented from the report because he believes that the estimate of damage to the inland forests is much

too low. Another member, Professor Paul Richards of the University College of North Wales, dissented for similar reasons. Professor Alexander Leighton of the Harvard School of Public Health, said that he couldn't go along with the conclusion because he has no expertise in the field. And Dr Meselson, who was a member of the report review panel, said last week that he believes that the report's estimate of tree damage understates the case by at least an order of magnitude.

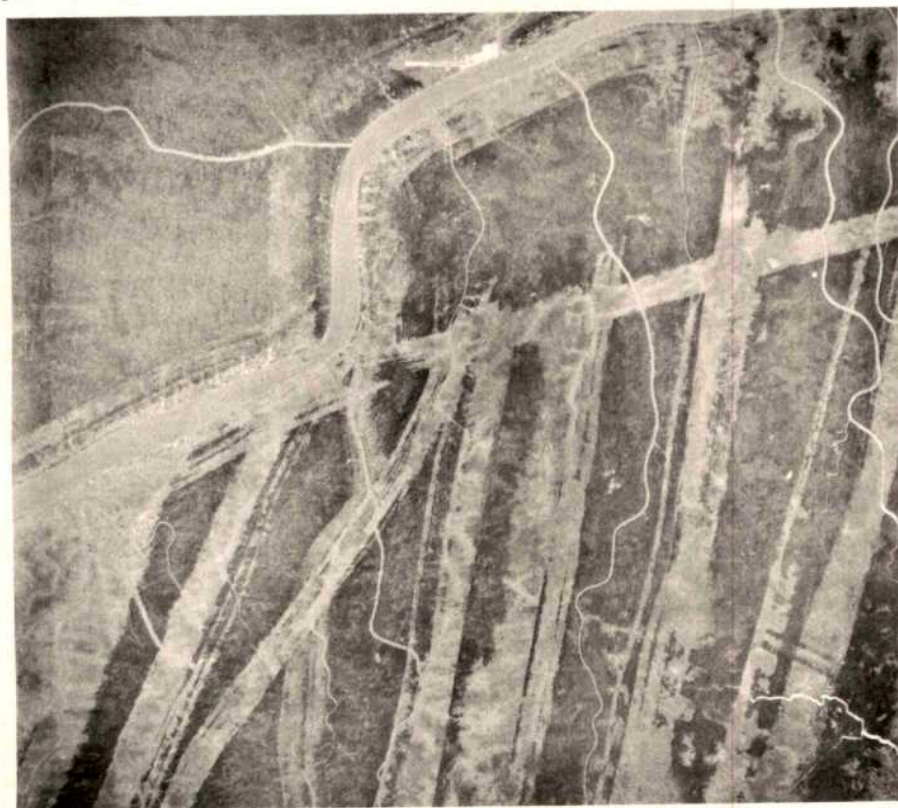
Meselson pointed out for example that the report gives a figure for the density of trees in Vietnam forests which is considerably less than the density of trees on the front lawn of the National Academy of Sciences—a lush, very lightly wooded expanse of grass. He said that this shows that there is clearly something wrong with the interpretation of the photographs, and he would like to see the academy team brought back together "if they can bear it" to take yet another look at the data. In any case, he said that he intends to publish his own conclusions independently.

Whatever the final outcome of the debate about the extent of the devastation of the inland forests, the academy report suggests that in lightly sprayed areas, redevelopment will probably resemble the pattern of forest growth following harvesting of large trees. The more heavily sprayed areas are, however, another matter, and will require considerable help in reforestation. It is reckoned that about 12% of the total sprayed area will require a major assistance programme, but Meselson believes that the figure is considerably greater.

As for the effects of herbicides on the people of Vietnam, the academy team looked for toxicity to people directly exposed to the herbicides, birth defects in the offspring born to exposed mothers, economic effects from crop destruction, and the psychological effect of the whole military herbicide programme on the Vietnamese people.

The most disturbing accounts of damage from acute toxic effects came from interviews with Montagnard people who live in the highland regions. The interviews, mostly conducted by Dr Gerald C. Hickey of the Southeast Asia Program of Cornell University, produced remarkably consistent reports of death to children, diarrhoea, skin rashes looking like insect bites, and abdominal pain following spray missions. They also reported widespread deaths among their animals, particularly chicken and pigs, and they said that the spraying killed fish in the streams.

Dr Hickey said in a telephone interview last week that he first heard about the reports of deaths from Montagnards in refugee camps, but he later went into



Aerial photograph of damage to mangrove forest in Ca Mau Peninsula in southern tip of Vietnam. Photo taken in 1971 shows effects of individual spraying missions over the forest. (Reproduced by permission of the National Academy of Sciences.)

the villages to talk to the people in their own localities. The reports, he said very accurately described the spray planes, the effects of the herbicides on the vegetation, and the accountants of the toxic effects on humans were consistent from village to village. The report itself says that "reports of illness following spraying are so striking it is difficult to dismiss them as simply the effects of propaganda, high normal death rates, or faulty understanding of cause and effect".

Asked why the reports of death came mostly from the highland people, Hickey suggested a number of possible reasons. First, they were hit mostly with agent orange, perhaps the most toxic of the herbicides used in the military operations, particularly since it is contaminated with TCDD. Second, the defoliation missions wiped out their crops because their fields are mostly in areas of reclaimed forest, and this tended to increase exposure to the chemicals. Third, the Montagnards tend to live out of doors much more than the lowlanders do, and they carry their children on their backs when they work in the fields. Thus they would be more likely to be exposed to the herbicide sprays. And finally, the people repeatedly emphasised in their interviews that they "didn't know what was happening" when the aircraft started spraying, and many of the children drank contaminated water or ate sprayed crops.

Since the reports came from direct interviews with Montagnards, they are difficult to verify by independent means, but Dr Hickey, a world renowned authority on the Montagnard people, is extremely disturbed by them. He is also extremely disturbed by Dr Handler's personal introduction to the academy report, which labelled the accounts as "secondhand" and "tales". Handler also said that the committee regrets that it was unable to visit the Montagnards in their own regions to verify the reports. Hickey did, however, get his information firsthand (albeit with the help of interpreters), and he did follow up his investigations in the Montagnard villages. He said last week that he had no chance to see Handler's introduction, in fact he didn't even know it would be written, and said that he finds it "outrageous". He said that in his nine years in Vietnam, he has encountered innumerable attempts at covering up the damage wrought by the war, and that this "is another one of them".

As for the effects of herbicides on birth defects and stillbirths, the report explicitly states that the team "could find no conclusive evidence of association between exposure to herbicides and birth defects in humans". The basis for that comment was a search through the records of two major Saigon hospitals

which showed "no consistent pattern of association between rates of congenital malformations and annual amounts of herbicides sprayed".

But a more extensive study is being conducted on the records of the Childrens Medical Relief International unit (the so-called Barsky Unit) of the Cho Ray Hospital in Saigon-Cholon. The study is not yet completed, but the report says that so far there is no strong evidence for an association between herbicides and birth defects.

Two members of an earlier survey of the effects of herbicides in Vietnam, the AAAS study, said last week, however, that the team had not conducted as thorough a study as it could, and suggested that a follow-up is required. Dr John Constable, of Harvard Medical School and Massachusetts General Hospital, suggested that the academy team had failed to look more closely at the figures from the Saigon Children's Hospital, particularly in the years following the AAAS study. "They reexamined our figures", he said, "but they have not attempted to add figures of their own".

The AAAS study pointed out that incidence of cleft lip, relative to other birth defects in the records of the Saigon Children's hospital, showed a marked increase in the years 1966-1968, the years of heaviest herbicide spraying. Although the AAAS team, which was led by Meselson, was careful to say that this effect was not necessarily correlated with herbicide spraying, the figures at least bear close scrutiny. The fact that the academy team had not looked at data from the hospital in the years after 1970 is, according to Meselson, "disappointing".

If there is a teratogenic effect from herbicide spraying, it is likely to have come from the TCDD contamination in the agent orange. TCDD—which is often more familiarly referred to as dioxin—is an extremely potent teratogen in mice, and when it was discovered in 1970 that it is always present in 2,4,5-T, there was great consternation about the risks to Vietnamese people exposed to agent orange. The chemical is such a potent teratogen that it is feared that amounts too small to be detected may prove to be harmful if they get into the food supply.

Last year, however, Meselson and his colleague Robert T. Baughman, reported an extremely sensitive method for detecting TCDD, which they used to analyse fish samples taken from South Vietnam in 1970. They found levels of the chemical ranging from 0.0007 parts per million to 0.008 parts per million. They are now in the process of analysing fish and shrimp samples taken last year, and preliminary results indicate that there is still some contamination with TCDD. The academy committee considered

these findings to be "a matter which warrants further attention". Use of Meselson's method for detecting dioxin in the food supply would be an ideal basis for selecting populations to study for birth defects.

Although the academy's study will clearly spark off a considerable debate about the conclusions that have been drawn, there is no doubt that it has added a huge amount of information on one of the most contentious issues to arise from the Vietnam war. In fact the report published last week was only a summary of the data (even though it ran to 400 pages), and the academy intends to publish later this year all the documentation gathered together during the three years that the report was in preparation.

It remains to be seen whether this wealth of information is sufficient to build up a strong enough case to convince the Administration that herbicides should be banned from use in war. The United States has yet to ratify the Geneva protocol of 1925 which outlaws the use in war of chemical weapons. Ratification of the treaty is now being held up because the US Senate insists that the protocol covers herbicides and tear gases, while the Administration insists that it doesn't. Secretary of State Henry Kissinger informed the Senate Foreign Relations Committee last year that the Administration's position is under review. The academy study is likely to play a considerable role in that review.

Unfilled places

MORE than six thousand university places, mainly in science, engineering and technology subjects, went unfilled in Britain at the beginning of the current academic year. The figures are contained in the eleventh report of the Universities Central Council on Admissions (UCCA), the body which handles the applications of most intending students in Britain. According to UCCA, the British universities expected to receive 71,875 students in October last year, and in fact only 65,586 places were taken. The shortfall in engineering and technology was 1,991, and in science 3,198. In a foreword to the report, Chairman of the Council Dr Geoffrey Templeman says that although the figures contain an element of estimation, and therefore need to be used with care, the picture they reveal is "not encouraging".

Science policy changes in Canada

David Spurgeon, Ottawa

GOVERNMENT announcements seem able to confuse as often as they can inform, and such was the case with those made recently about the forthcoming changes in federal science bodies in Canada.

The announcements came in the Speech from the Throne at the opening session of Canada's 29th Parliament on February 27, and in press releases issued about the same time by the Ministry of State for Science and Technology (MOSST). Those in the throne speech informed only in the briefest and most general way, while the details offered by the ministry's press releases served mostly to confuse.

The throne speech said: "the Ministry of State for Science and Technology will be developing national science objectives as a basis for exercising enhanced advisory and co-ordinating authority within the government. Two new granting councils will be formed, one for social sciences and humanities and the other for natural sciences".

The ministry's press releases spoke in different terms: "the government plans to strengthen the Ministry of State for Science and Technology in order to ensure a more efficient use of human resources and scientific activities in the pursuit of national goals", said one. "Its (the ministry's) advice will increasingly be taken into account by Cabinet, in relation to new science oriented policies . . ." said another, labelled statement by Mme Jeanne Sauve, Minister of State for Science and Technology. "Its impact will also be markedly increased in relation to the assessment of scientific programs and associated expenditures. Close cooperation between the ministry and Treasury Board is developing in these areas and it is the intention to focus attention, not only within the government itself, but also in the public domain on the distribution and extent of expenditures in science".

The question in the minds of some was whether the changes outlined meant that the ministry was finally becoming what they had feared it might become—an all-powerful monolithic instrument of power in Canadian science policy—or whether its statements only gave that impression.

That they did give that impression was confirmed by a quick sampling of early reactions among scientists who had read the press releases or news stories about them. Such reactions ranged from expressions of fear and concern to outright predictions of gloom and doom for the future of science in Canada. The

doomsters saw the moves as a political power play by the fledgling ministry, which they feared would create a super-bureaucracy concerned with administrative efficiency and political expediency rather than the good of science or its judicious application.

But those closer to the federal bureaucracy saw things differently. The realities of the situation meant that such a consolidation of power by the ministry was impossible even if desired, they contended, and the politics of the scientific establishment would assure that there is no cause for apprehension.

The changes outlined clearly in the science ministry's press releases were these:

- The granting function of the National Research Council will be separated from the NRC laboratories and removed to a new council entitled the Natural Science Research Council.

- The granting function for the social sciences and the humanities will be removed from the Canada Council and established in a new granting council to be called the Social Sciences and Humanities Research Council. The Canada Council will continue to remain responsible for support of the arts.

- The Medical Research Council's granting functions will remain undisturbed, but the MRC will be placed into "an appropriate relationship" with a new Inter-council Co-ordinating Committee.

- This new Inter-Council co-ordinating Committee will be established by the government to advise on allocation of funds among the granting councils; ensure coverage by the councils of all recognised disciplines; standardise granting practices; ensure that the needs of inter-disciplinary research are met; and coordinate and advise on council programmes—as well as those of federal government departments—in support of university research.

- The new committee will be chaired by the Secretary of the science ministry and will report to the Minister of State for Science and Technology.

- The science ministry will be involved in development of a science policy framework to provide guidance on scientific activities to all departments and agencies in terms of science objectives, priorities and strategies; the development of science expenditure guidelines for science agencies and the Treasury Board; for collaborating with the Treasury Board in analysing expenditures and programme proposals; and in annually assessing accomplishments of government science activities.

Some saw in these moves control by the ministry of the granting agencies and a pre-eminent position for the ministry in directing the use of fiscal sup-

port for science. This seemed to be confirmed by further announcements that the Science Council of Canada Act would be amended to give the council a more public role, while the ministry would take over its former role as official science advisory body to the federal government.

The announcements were also seen as evidence that the government had taken seriously the recommendations of the Senate Special Committee on Science Policy (the so-called Lamontagne Committee), which in its report No. 3 had said that the science ministry's advisory role was not good enough for its survival, and that "sooner or later MOSST will face a dead end unless it is given more authority . . . the committee believes that the Ministry of State for Science and Technology should become the focus of a central machinery for the concentrated planning and control of government involvement in science and technology".

Where the confusion arose was in certain ambiguities—and certain omissions—in the ministry's press releases. For example, while the press releases referred to the past reporting practices of the NRC and MRC and Canada Council, they avoided saying explicitly where these agencies might report in future, while making it easy for the reader to conclude that this would be, in effect, to the Minister of State for Science and Technology through the new Inter-council Co-ordinating Committee.

In fact the NRC and MRC are both Crown corporations and the Canada Council is a foundation, which report to Parliament through the President of the Treasury Board (NRC), the Minister of National Health and Welfare (MRC), and the Secretary of State (Canada Council). Changes in legislation would be required to change this, and nothing in the throne speech presaged such changes. The heads of the MRC and Canada Council are known to understand that their respective reporting procedures will not be changed. And it seems illogical that a humanities council head would report to the science minister, though the ministry press releases are silent on this point. Nor do they say to whom the new Natural Sciences Research Council will report.

Equally vague are the references to the relationships of the granting councils to each other and to the science ministry as members of the new co-ordinating committee. Even the make-up of the committee is left a mystery: it "will include in its membership, the heads of the granting councils and certain other senior officials to be named later".

Old Ottawa hands see all this as a struggle for power by the science ministry, but a struggle with an extremely uncertain outcome. Concerning the con-

tinued autonomy of the granting councils, which is seen as crucial to the future of science in Canada, a great deal will depend on the way the Inter-council Coordinating Committee works, and this in turn depends greatly on its membership and the effectiveness of its chairman.

The possibility of the ministry using its enhanced position as government science adviser and budgetary watchdog as a path to massive centralisation of power is also seen as unlikely, given the realities of the situation. The science minister is not a member of the Treasury Board and has not the political heft of huge science-related departments like Environment. Nor need the Treasury Board heed the ministry's advice unless it finds it good—it has its own advisers as for other areas, and conducts its own assessments, and will continue to do so. Or so runs the argument.

Whatever the outcome, some observers are wondering what happened to another major recommendation of the Lamontagne report (and one made by other studies): the need for a better linkage between government R&D and industry. None of the re-organisational changes seem bent toward this goal—and in fact the splitting of NRC's laboratories from the granting council (which provided such a link) seems actively to work against it. The NRC laboratories, it seems, will be isolated.

Velikovsky in the open

Miranda Robertson, San Francisco

THE scientific session on the heterodox theories of Immanuel Velikovsky at the meeting of the American Association for the Advancement of Science (AAAS) in San Francisco on February 25 was the first and probably the most serious of four symposia to be held this year on various issues raised by Velikovsky's ideas and his attempts to propagate them. The organisers of the AAAS symposium adopted the stance that Velikovsky has offered a 'challenge to science'. Science, in the persons of Dr P. J. Huber (Swiss Federal Institute of Technology, Zürich), Dr J. D. Mulholland (University of Texas) and Dr C. Sagan (Cornell University) rose to meet it.

The only remarkable thing about the resulting session was the amount of time and energy Drs Huber, Mulholland and Sagan had been prepared to put into it. There seems little danger that any real scientist or scholar would be tempted to take Velikovsky's ideas seriously. Best-selling books are usually fiction, and Velikovsky's *Worlds in Collision*—

published in 1950 by Doubleday after the Macmillan Co., showing more academic integrity than business acumen, had passed it on to them—is plainly no exception. Velikovsky himself is by birth a Russian Jew trained in medicine and psychiatry.

Ostensibly, the fundamental issue at the symposium was Velikovsky's version of the birth of Venus. His story is that the planet originated as a comet torn out of Jupiter about 1500 BC, when it went into an eccentric orbit in which it narrowly missed the Earth a number of times and disrupted the orbit of Mars before settling down about 800 BC in its present position in the solar system. All this is documented in the Bible (the seven plagues of Egypt, the Israelites' manna from Heaven and various other miraculous phenomena were the consequences of cosmic catastrophe) and in some ancient Egyptian writings.

Huber was brought in to deal with Velikovsky's Egyptology and Assyriology, Mulholland to tackle his celestial mechanics, and Sagan to do battle energetically on all available fronts at once. Velikovsky, however, turned out to be too easy to discredit. He muddles his myths, confusing Athene and Aphrodite; and his chemistry, confusing carbohydrates with hydrocarbons—so that Venus at once bestows upon the Earth supplies of petroleum (hydrocarbon) and manna for the children of Israel (carbohydrate) from its atmospheric clouds, now known in any case to consist of sulphuric acid. The spin resonance of the Earth with Mars and Venus can only be explained by the stability of all three planets in orbit in the solar system for billions of years. Cuneiform texts reliably dated well before 1500 BC refer to a Venus behaving recognisably as Venus behaves today.

Velikovsky replied to his critics with rhetoric not reason. The issue, in short, was not scientific but sociological. Indeed it was stated at the beginning of the session by Dr N. Storer, who is a sociologist at the City University of New York: in principle, the progress of science depends on the patient and impartial evaluation by the scientific community of all new ideas and work. In practice, no-one has time to examine the arguments of every heterodox claimant to new insights and it is surprisingly easy to be certain in the majority of cases that one is not withholding recognition from a second Einstein.

Velikovsky, however, claims not merely to have been ignored but to have been unfairly attacked by the scientific establishment. This was the real burden of his challenge, and the attempt to deal fairly and seriously with his thesis succeeded in illustrating exactly what makes scientists lose patience with the majority of far-reaching new theories. Generally,

they are so loosely stated as to present inadequate premises for logical or quantitative reasoning. This point was emphasised by Dr Sagan, both explicitly and implicitly in his struggle to find the substance of Dr Velikovsky's theories.

For Sagan—himself no mean propagator of wild theories, as might be inferred from his editorship of the journal *Icarus*—the professed object of the apparently unedifying exercise in San Francisco was the demonstration of the essence of scientific method, and what happens when its criteria are applied to fantasies such as Velikovsky's.

Actually, like the symposium on unidentified flying objects held by the AAAS five years ago, it was an exercise in setting the record straight. The sensational speculations are made available to everyone by the popular press, but where are people to find the sober evaluations? Sagan has suggested that this should be an occasional but regular function of organisations such as the AAAS. In many ways this seems a more plausible justification for unweildy annual science meetings than the pretence that they do anything to further the cause of science. Furthermore, the subject matter for the meetings would thus be selected by an entirely democratic process, since the only criterion for including a subject in the agenda for discussion would be the amount of public interest it had aroused. Whether the public actually wants to be disabused of sensationalist notions is, of course, another matter.

Seeing double

ANOTHER curious piece of programming by the BBC on Monday March 4 produced "Horizon" on BBC2 telling us that "The Future Goes Boom!" immediately followed by "What on Earth... are we doing?" on BBC1 in which Dr David Bellamy told us that we might just struggle along if we all tighten our belts. A more fruitful approach might have been to confront Professor Hermann Kahn, the prophet of boom, with Bellamy to thrash out the issues. As things stand, the protagonists are about even; both programmes opened with film of an Apollo launch, but the print used by the "Horizon" team was much sharper and in better colour. To balance this, Bellamy's subsequent down-to-Earth approach made more sense than Kahn's frenetic globe trotting while calculating, apparently on the back of the proverbial envelope, just how many people are likely to be killed in the forthcoming nuclear war. But a solid 2½ hours of proselytisation with no lucid discussion or pause for thought probably turned most viewers completely off, if they did not first turn it off.

news and views

When the lunar crust formed

THREE distinct aspects of lunar history have been dated from age determinations of rocks from the Moon. The basaltic lavas filling the visited maria sites have ranged in age from 3,100 to 3,900 million years. These lavas were produced by melting or partial melting of rock in the interior of the Moon by heat generated by the radioactive decay of uranium and thorium. The implied absence of younger rocks over a substantial part of the lunar surface is explained in part by the concentration of radioactive heat sources in the outer regions of the Moon which, coupled with the smaller size of the Moon, makes for a more efficient heat loss than is the case for the Earth. Internal motions within the lunar interior have been unable to disrupt the relatively thick crust and in consequence there have been no large scale horizontal movements of the crust to assist the process of continuing lava extrusion, again in contrast to the Earth. In general the lavas have been extruded into large, impact-generated basins, possibly along lines of weakness related to the impact.

An earlier episode of lunar history is represented by the dating of the complex fragmental rocks from the various highland sites. The ^{40}Ar - ^{39}Ar and Rb-Sr (mineral isochron) ages of highland rocks are, with a few exceptions, confined to a narrow time interval from 3,900 to 4,000 million years, indicating that the rocks were last at elevated temperatures at this time rather than that this is when their individual components crystallised. The ages have been taken by several authors to indicate the times when a number of the large impact basins were formed but there is no consensus as to how many basin-forming events are represented in the ages, and estimates vary between two (Orientale and Imbrium) and six (Orientale, Imbrium, Crisium, Nectaris, Humorum, Serenitatis). All authors are agreed that the first 500 million years of lunar history has been obscured by the intense bombardment of the highlands.

Evidence for the earliest period of lunar history has so far come from a comparison of the isotopic composition of Sr and Pb in suites of rocks from the various sites, in the lunar soil, and in one particularly exotic rock, 12013, from Apollo 12. All of these rocks have been subjected to reheating or melting at or more recently than 4,000 million years ago, but it is possible to draw inferences about the isotopic composition of the precursor material and specifically to show that regions of distinct chemical composition (differing Rb/Sr and U/Pb ratios) existed as early as 4,500 million years ago. The physical implication of this is that melting and the separation of chemically distinct rock types must have begun as early as 4,500 million years ago—shortly after the Solar System formed. The heat source responsible for this early melting is not clear although the gravitational potential energy released in accretion would be adequate if accretion were sufficiently rapid.

In the article on page 199 of this issue of *Nature*, Jessberger, Huneke and Wasserburg have used a third technique, the ^{40}Ar - ^{39}Ar method, to find evidence of relict crustal material in a 4,000 million-year-old breccia. The ^{40}Ar - ^{39}Ar method is a modified form of K-Ar dating first applied at the University of Sheffield to the dating of lunar samples from Apollo 11. It is a neutron activation technique which con-

verts some of the potassium in the rock to ^{39}Ar , and the age can be determined from the ($^{40}\text{Ar}/^{39}\text{Ar}$) ratio in the gas extracted by heating the sample at a succession of increasing temperatures. The method is in principle able to detect variations in the ($^{40}\text{Ar}/\text{K}$) ratio which could result from partial argon loss in a sample with a complex thermal history and in suitable circumstances to unravel that history.

Jessberger, Huneke and Wasserburg have applied the technique to separated minerals from a highland breccia which previously had been shown by Rb-Sr measurements to have been heated 3,980 million years ago but to temperatures insufficient to homogenise the Sr isotopic composition. The ($^{40}\text{Ar}/^{39}\text{Ar}$) ratios measured in a heating experiment of 30 to 50 steps correspond for the most part to this age of 3,980 million years, apart from small variations which seem to be an artefact of the technique, possibly related to the recoil of ^{39}Ar during neutron activation. In the highest temperature extractions from a sample of the plagioclase which showed the anomalous Sr isotope composition, however, the ($^{40}\text{Ar}/^{39}\text{Ar}$) ratio increased to a value corresponding to 4,500 million years. This would seem to indicate that when the breccia went through the heating episode at 3,980 million years most of the preexisting ^{40}Ar was driven off, except in some very retentive crystal sites in the plagioclase, and that the argon in these sites is retaining a memory of when the plagioclase originally crystallised as part of the early lunar crust, 4,500 million years ago. Thus all of the radioactive clocks now provide independent evidence that the crust of the Moon was beginning to form a very short time after the Solar System itself formed.

G.T.

From genes to behaviour

THERE is many a potential intermediary process twixt the gene and the behaviour it affects. This has been a general problem facing behaviour geneticists as they search for clues on the mechanisms of gene action. Sometimes the answer has been boringly obvious—the mutant mouse which builds nests larger than normal turns out to have thinner fur, and so on. But a number of the older studies on genes affecting nest cleaning behaviour of honeybees (Rothenbuhler, *Am. Zool.*, 4, 111–123; 1964) or courtship displays of *Drosophila* (Bastock, *Evolution*, 10, 421–439; 1956), for example, revealed effects which could reasonably be explained only by action on the central nervous system. There is, of course, no shortage of neural elements as potential sites for primary gene action, and for complex behaviour patterns it is difficult to take the story further.

The past few years have seen some important developments in behaviour genetics, relying on the identification of behaviourally-effective mutants induced in *Drosophila* and nematodes. The philosophy behind such work contends that behaviour genetics must be built from the bottom upwards, concentrating first on the simple behavioural repertoire of organisms with simple nervous systems.

Brenner and his group at Cambridge have progressed further than most with their nematode *Caenorhabditis elegans*, which has a nervous system of only a few hundred elements. A series of mutants which affect the worm's locomotion have been identified and some of these produce detectable and pre-

cisely regular defects in neural interconnectivity. Some of these defects are confined to one or two neurones whereas others affect every one of a series along the worm's body.

The regularity of nematode structure allows a finer-scale of analysis than is yet possible with *Drosophila*. Hotta and Benzer (*Nature*, **240**, 527-535; 1972) have used an elegant technique involving genetic mosaics to localise the site of action in their *Drosophila* behavioural mutants. They can definitely ascribe some of these to the brain alone, but so far only Ikeda and Kaplan (*Proc. natn. Acad. Sci. U.S.A.*, **66**, 765-772; **67**, 1480-1487; 1970) have pinned down a neural site precisely. One of their *hyperkinetic* mutants, which shows abnormal leg movements under ether anaesthesia, has modified spontaneous activity in specific motoneurones lying laterally in the thoracic complex. Not only have Ikeda and Kaplan been able to isolate these neurones from inputs and show that they retain the abnormal pattern, but they have used the most perfect control for systematic effects—a left/right mosaic in which the normal left half of the body can be compared with its mutant right.

On the sensory side Benzer's group, and others, have isolated several mutants which affect the electroretinogram response of the *Drosophila* eye. Most of these were picked up by screening the responses of large numbers of flies to various visual stimuli. Eyes are complex and mutants affecting the chemical senses have the advantage of a much simpler neural substrate. Ward (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 817-821; 1973) has already described some genes affecting the chemotactic responses of *Caenorhabditis*. Kikuchi (*Nature*, **243**, 36-38; 1973) found a dominant in *Drosophila* which caused flies to be attracted by chemicals which repelled normal individuals. Now in this current issue (page 243) Isono and Kikuchi describe another autosomal recessive with a specific effect on the chemosensory hairs of the labellum (and presumably also those on the tarsi) which respond to sugars. In particular the mutation greatly reduces the response to glucose. Such a mutant should prove useful for the identification of receptor sites for sugars at the hair tip.

Slowly a catalogue of behavioural mutants is being built up. The bricks are few and the wall to be constructed is of enormous dimensions, but soon one may be able to detect some pattern in the brick laying. Nevertheless, those working with complex behaviour will have to wait some time for explanations of behavioural potential in genetic terms.

A.M.

Capping related to type of cell

TREATMENT of lymphocytes at 37° C with low concentrations of reagents such as concanavalin A or anti-immunoglobulin antibody leads to movement of the binding sites into large patches known as 'caps'. It now seems that the situation established previously is by no means a biologically homogeneous event. Stackpole, Jacobson and Landis show convincingly on page 232 of this issue of *Nature* that the exact location on the cell surface at which a cap forms depends in a critical and as yet undetermined manner on the type of cell and to some extent on the particular surface molecules being capped. Caps can form over or opposite to the Golgi region of the cell. Pinocytotic vesicles are seen only when the cap is formed over the Golgi region. Furthermore the new results may help clarify several puzzling and conflicting observations of the effects of drugs such as cytochalasin B on cap formation.

Caps are formed in the classical way by first exposing mouse thymus or spleen cells to specific mouse alloantibody directed against a particular surface membrane antigen and this is followed by cross linking with anti-mouse immuno-

Are supernovae disruptive?

It is arguable that all supernova explosions occur in binary systems, as a result of mass transfer between close companions. It is also arguable that all pulsars are created in supernova explosions. So the lack of any evidence to show that any pulsar is a member of a binary system looked at first sight embarrassing, and led in turn to the suggestion that supernovae always disrupt the binaries which give them birth. But the more recent discovery of X-ray 'pulsars' which are members of binary systems has encouraged a retreat from this extreme position; on page 208 of this issue of *Nature* Sutantyo shows that present theories of stellar evolution do indeed suggest that supernovae occurring in close binaries need not be disruptive in all cases.

It is interesting to speculate that this work may form the basis of a theoretical understanding of why some binaries should explode to produce variable X-ray sources, whereas others are turned into pulsars. As Sutantyo points out, the known X-ray binaries each contain an OB supergiant with mass some tens of solar masses, with a companion of around 0.5 to 5.0 M_{\odot} ; the large mass ratio suggests that the explosion occurred before mass transfer. In that case, the less massive star is probably the one involved in the explosion, and all such binaries with periods larger than 5 d remain bound after the explosion. That is more than 50% of all such systems.

Perhaps, as Sutantyo suggests, the only reason that radio pulses are not visible from such binaries is that the radio emission is absorbed and smeared out by the gas flows between the two stars. Any remnant which is left on its own by a disruptive explosion cannot remain an X-ray source for long, since this energetic emission requires a constant input of gravitational energy released by mass transfer between the components, according to the best theories. So such unattached remnants would inevitably be seen as radio pulsars, if they were seen at all.

And that, of course, raises the question of whether such sources are merely the minority cases from the explosions considered by Sutantyo (which seems unlikely in view of the number of pulsars known) or whether other kinds of binary system are indeed more often disrupted than not as a result of supernova explosions. Certainly there is more than one kind of supernova; do the different kinds produce different offspring?

J.G.

globulin antibody. Stackpole *et al.* used hybrid anti-mouse IgG/anti-ferritin antibodies that are revealed on the cell surface by adding ferritin. In this way it was found that a very high proportion of spleen cells display a cap of ferritin and this indicates, of course, a similar distribution of the underlying cell surface antigen. Almost without exception the caps were located over the Golgi elements of the cell when surface immunoglobulin molecules or concanavalin A receptor sites were capped. By contrast capping of H-2 antigens on mouse spleen cells is not polarised but patches form over and opposite to the Golgi region. A clear difference is seen with mouse thymus cells compared with spleen cells: in the first place Thy-1 and TL alloantigens capped predominantly over the Golgi region in spleen cells, whereas the Thy-1 alloantigen of thymus cells, for instance, is moved into the opposite pole of the cell.

It was shown previously by several groups that cap formation is often accompanied by pinocytosis of labelled membrane. After a relatively short period at 37° C cells may have much of their ferritin label within the cytoplasm and attempts to relabel the surface with reagents specific for the internalised receptors give negative results. It seems that pinocytosis takes place only in the special case in which caps form in the vicinity of the Golgi apparatus. Thus, caps of Thy-1, surface immunoglobulin or concanavalin A binding sites on mouse spleen cells are extensively internalised. In thymus cells very little pinocytosis of labelled surface markers takes place.

Stackpole *et al.* can distinguish further the two types of capping by the effects of certain inhibitors. General inhibitors such as sodium azide (and probably dinitrophenol) inhibit capping of all antigens both in spleen and thymus cells, but cytocholasin B has an effect only on the movement of antigens towards

the pole of the cell immediately adjacent to the Golgi apparatus. The drug therefore has almost no effect on capping of H-2 antigens on spleen cells, and redistribution of thymus cell surface markers is also not inhibited. In agreement with earlier studies, reagents affecting cellular microtubules, such as colchicine, vinblastine and colcemid, do not inhibit either type of capping. Indeed according to Berlin *et al.* (*Nature*, 247, 45-46; 1974) colchicine in certain conditions even facilitates capping of concanavalin A receptors. The effect of cytocholasin B, however, does suggest that at least one form of capping is actively dependent on intracellular contractile elements.

The way in which the direction of capping is controlled for a particular surface marker—for example H-2 antigens in spleen cells—remains to be determined. Clearly, the situation is very complex and many cellular processes providing multiple target sites for drugs are probably involved. R.C.H.

Translational repression

from a Correspondent

It is well known that the coat protein of group I and III RNA bacteriophages can act as repressors of the synthesis of the replicase by binding to their homologous RNA. Three reports in *Nature* this week (see pages 204, 223 and 225) by Steitz and two groups of collaborators shed fascinating light on the details of this process. Two of the papers are concerned with the secondary structure of the fifty-nine nucleotide-long segment of R17 RNA which is protected against T_1 nuclease attack by the coat protein and which has been sequenced. It contains the end of the coat protein gene including the tandem terminator and extends through the intercistronic region to the initiator region of the following replicase gene. Inspection of this sequence has suggested the presence of two helical regions, A and B; the first involves the coat protein terminator and the second the replicase initiator region.

Gralla, Steitz and Crothers from Yale have studied the melting behaviour of this fragment and demonstrate that differential melting curves exhibit two sharp peaks indicative of two substantial helical regions. Analysis of the details of these peaks allows estimations of the T_m and enthalpy of melting of these two helices. These estimations are in good agreement with the values calculated for the two helical regions A and B by methods worked out by the same group and assuming that helix B melts at the lower temperature.

This assignment of the lower melting peak to helix B is confirmed by the

work of Hilbers, Shulman, Yamane and Steitz from the Bell Telephone Laboratories who have studied the nuclear magnetic resonance (NMR) spectrum corresponding to the ring NH protons which are involved in the formation of A-U or G-C pairs. Their data suggest that at 26° C there are sixteen base pairs of which eight are no longer apparent at 40° C. A detailed analysis of the NMR spectra in terms of sequence-dependent ring current shifts suggests that the eight base pairs that are lost on increasing the temperature are derived from helix B. The disappearance of the signals corresponding to these base pairs does not necessarily imply that the helix is melted completely by 40° C but only that the mean lifetime of a base pair is less than about 5 ms. Indeed the data of Gralla *et al.* suggest that both helices are essentially intact for most of the time at 25° C in the presence of moderate concentrations of cations.

Gralla *et al.* went on to study the kinetics of the melting processes by temperature jump techniques. These measurements give time constants for these processes and also their amplitudes. As the temperature was increased from 25° C, a rapid process was observed with an amplitude that increased to a maximum at the lower T_m . Thereafter this amplitude decreased and was replaced by a slower process with an amplitude that increased to a maximum at the higher T_m . Clearly these two processes reflect the independent melting of two helical regions.

In further studies, Gralla *et al.* found by sedimentation equilibrium measurements that the molecular weight of a complex of the coat protein and this fragment reached a plateau value for a 1:1 mixture. Melting studies of this 1:1 complex revealed that although the T_m of neither helical region was altered by binding of the coat protein, the rate of melting of the lower melting helix B was reduced by a factor of 100 while that of helix A was unchanged. After considering various alternative hypotheses, these authors favour an interpretation that involves the binding of helix B to the coat protein and some irreversible process, perhaps involving the denaturation of the protein at the T_m of the helix.

That the coat protein recognises the B helix is further confirmed by the work of Steitz in the third paper who found that the coat protein will also bind to a twenty-nine nucleotide-long portion of the larger fragment which incorporates the B helix but not the A. Furthermore, an oligonucleotide from the turn of the B helix will not bind efficiently and this suggests that the B helix itself is recognised by the coat protein. Steitz points out that the sequestration of the initiator region in the B helix could account for the inability of *Escherichia coli* ribosomes to recognise the replicase initiator region.

It is emphasised by the authors of these papers that these helical regions may not exist in the intact R17 RNA at all times, especially when ribosomes do succeed in recognising the replicase initiator region.

Ice cover and global weather shift

by our Cosmology Correspondent

"Snow and pack-ice cover in the northern hemisphere formed earlier in the year and covered a larger area in the past 3 years than it did 7 years ago, when systematic satellite mapping began. This shift, in all likelihood, has produced a significant change in the hemispheric heat balance. The difference was most pronounced in the fall and was especially large in 1971. The anomalous global weather patterns of 1972 and 1973 may be the result of these developments."

That is the conclusion reached by G. J. and H. J. Kukla after studying weekly maps supplied by the US National Oceanic and Atmospheric Administration (NOAA) and based on photographs obtained by meteorological satellites. All snow and ice fields lasting more than 5 days are recorded on these photographs. The dramatic implication of this work is that with a better understanding of how the Earth's atmosphere

works, the changes which have caused such trouble in the northern hemisphere recently (and indeed those which are causing trouble in Australia now) might have been predicted in time for effective action to be taken.

As Kukla and Kukla point out (*Science*, 183, 709; 1974), the location and duration of snow and pack ice fields are the most important seasonal factors which affect the Earth's heat balance. Because of the heat required for melting snow and ice, peak air temperatures in summer at mid-latitudes are delayed after the peak insolation. In the northern hemisphere, permanent ice covers some 10×10^6 km², and seasonal ice and snow covers a further 50×10^6 km²; in the south, the corresponding figures are 14×10^6 km² and 20×10^6 km². The 'temporary' cover in the north is greater, of course, because of the presence of continents on which the snow can lie. Satellite mapping provides the only reliable means of monitoring the changing ice patterns over a whole hemisphere.

These studies are facilitated by dividing the year into four snow cover (SC) seasons. SC summer is the part of the year when less than 15×10^6 km² of

the northern hemisphere are covered; SC winter the period when more than 55×10^6 km² are covered. During the past 7 yr, SC winter and SC summer have been about the same length, averaging 80 to 90 d. But SC spring was, on average, about 7 weeks longer than SC fall. And the date on which SC fall and winter started were earlier in each of the successive years 1971, 1972 and 1973. In 1971, the change was dramatic, with SC fall starting 3 weeks earlier than in any of the 4 preceding years.

During the same period, there was a gradual increase in snow and ice cover found during the October/November period. For the entire 7 yr of this study, the average annual coverage was 34.9×10^6 km²; in 1971 alone this increased by 12% and since 1971 the average has fluctuated about a new mean of 36.9×10^6 km². On November 1, 1973, the cover was 36.7×10^6 km². So the period since 1967 divides naturally into two distinctly different sections.

Kukla and Kukla discuss at some length the possible sources of error in their study, and argue a convincing case that the effect they report is real. "There is no doubt", they say, "that the surface heat exchange in 1971 must have dropped

markedly". It is still too early to say definitely that this caused the change in global circulation which has, in recent months, been the subject of a great deal of discussion both in these pages and elsewhere; but it may well be significant that the changes in ice cover occurred before these changes in circulation became apparent. It seems plausible, at least, to argue that the observed changes in mean temperature and other parameters during the past 30 yr eventually produced the increased snow cover which has in some way disturbed the atmospheric circulation.

Superfluid ³He confirmed

from our
Condensed Matter Correspondent

THE report in a recent issue of *Physical Review Letters* (32, 141; 1974) by Kojima, Paulson and Wheatley of the University of California at San Diego that they have observed the propagation of fourth sound in the two newly discovered phases of liquid ³He amounts to the first unequivocal evidence that both of these new phases are superfluids.

It has long been known that liquid ⁴He is superfluid below its transition temperature at about 2 K but, until 1972, it was part of the conventional wisdom that the other isotope, ³He, was not a superfluid. This was mainly because of the theoretical impossibility that liquid ³He, whose atoms contain an odd number of fundamental particles and are called fermions, could become superfluid in the same manner as liquid ⁴He by undergoing a Bose-Einstein condensation; this phenomenon can only occur in assemblies whose components, bosons, contain an even number of fundamental particles. It was realised, however, that the electrons (also fermions) in a superconductor, at that time the only other known superfluid, acquired their superfluidity in a somewhat different way from ⁴He; and it had been suggested that a transition analogous to the superconducting transition in metals might occur in liquid ³He at a sufficiently low temperature, leading to some sort of superfluid state.

The first indication that this suggestion might indeed have been correct came some fifteen years later when Osheroff, Richardson and Lee of Cornell University discovered that two quite separate phase transitions occurred in a cell containing a mixture of liquid and solid ³He when it was cooled under pressure below 3 mK (*Phys. Rev. Lett.*, 28, 885; 1972). In subsequent experiments, Alvesalo, Anufriyev, Collan, Lounasmaa and Wennerström of the Technical University at Helsinki used

Synthesis *in vitro* of silkworm ecdysone

from our Insect Physiology Correspondent

TWELVE months ago some of the queries that have arisen concerning the site of synthesis of ecdysone in insects were discussed (see *Nature*, 242, 86; 1973). Briefly, the problem has been that, in spite of the conclusive experimental evidence (provided originally by Fukuda) that the moulting factor of insects is produced by the prothoracic glands, it has not proved possible to get convincing proof that the product ecdysone as isolated by Karlson and Butenandt is synthesised by these glands. Only the most minute amounts of ecdysone from the prothoracic glands have been extracted, and glands maintained *in vitro* produced only insignificant amounts of the hormone.

A group of Japanese workers led by Chino (*Science*, 183, 529; 1974) have now reinvestigated this problem and have found, as earlier workers had done, that the isolated prothoracic glands of the silkworm *Bombyx* maintained in the culture media of Grace or of Wyatt produced only minute amounts of ecdysone (less than 5 ng per pair of glands). Unchanged haemolymph blackens and is an unsatisfactory medium; but by isolating the haemolymph proteins on a Sephadex column and suspending these in Wyatt's culture medium and saline Chino and his colleagues attained a far more satisfactory "haemolymph medium". Arguing that the rich tracheal

supply of the glands indicated a high oxygen requirement for synthesis, they exposed the gland pairs, each pair in a single drop culture, to oxygen instead of air. By these combined procedures the hormone produced per gland pair increased in amount from 5 ng to 120 ng.

It is well known that the insect requires cholesterol for ecdysone synthesis. The authors therefore concluded that the lipoproteins of the haemolymph are likely to be the essential component so they then purified the two chief lipoproteins I and II from the haemolymph and prepared a culture medium by addition of these to Wyatt's medium—and this proved even more effective than the haemolymph medium. The hormone synthesised by the glands is at once liberated to the medium; in agreement with earlier observations, almost no ecdysone can be extracted from the glands themselves. Thin layer and gas chromatography established that the hormone produced by the glands is solely α ecdysone; as had already been suspected the more active and more polar product, β ecdysone must be formed from α ecdysone elsewhere in the body. Chino *et al.* report that another research team in the laboratory of Gilbert, working independently, has obtained similar results with the prothoracic glands of the tobacco hornworm *Manduca sexta* (*Proc. natn. Acad. Sci. U.S.A.*, in the press).

a vibrating wire viscometer to show that the viscosity of the liquid fell rapidly with decreasing temperature below the higher temperature transition in the so-called A phase of the liquid, and even more rapidly in the B phase below the second transition (*Phys. Rev. Lett.*, **30**, 962; 1973). This result strongly suggested the onset of superfluidity but the evidence was not conclusive in that the experiment did not demonstrate unambiguously the presence of any fluid with zero viscosity.

Fourth sound is a phenomenon well known in the case of liquid ^4He , which can be regarded as an homogeneous mixture of normal and superfluid components; it is a wave motion which can occur when the liquid is absorbed into a tightly packed powder. In these conditions the normal fluid component, which has a finite viscosity, is effectively immobilised but the superfluid component, with zero viscosity, is still entirely free to move. The pressure wave which can thus be transmitted through superfluid in the interstices of a tightly packed powder is known as fourth sound; and the densities of the normal fluid ρ_n and of the superfluid ρ_s can be deduced from measurements of its velocity. This wave mode can exist only if some superfluid is present, so it was natural to search for a possible fourth sound mode in each of the new phases of liquid ^3He .

The experimental cell used by the San Diego group was constructed from an epoxy resin and was tightly packed with finely ground crystals of the paramagnetic salt cerium magnesium nitrate (CMN). Pressure transducers were positioned at either end of the cell so that fourth sound, if it existed, could be both excited and detected. The function of the CMN was threefold: its adiabatic demagnetisation enabled the liquid ^3He trapped in the interstices to be refrigerated to the required temperature; it acted as the superleak in which the fourth sound could be excited; and the temperature could be deduced from measurements of its paramagnetic susceptibility. In the event it was found that fourth sound could indeed be propagated, not just in one, but in both of the new phases, proving beyond doubt that both are superfluids.

The velocity measurements were used to derive values of ρ_s , which turned out to be surprisingly low: much lower, in fact, than may be deduced from the Helsinki viscosity experiment. The reasons for this apparent inconsistency are not yet clear. The San Diego group admit, however, that their values of ρ_s may perhaps be strictly relevant only to liquid trapped in fine pores, since it is known that for ^4He under similar conditions ρ_s is somewhat smaller than in the bulk liquid. Be this as it

may, the group's experiment has now confirmed what many physicists had come to believe would probably turn out to be the case: that, with the discovery of the new phases of ^3He , the number of known superfluids in nature has been doubled.

Resolving conflicts by time sharing

from our *Animal Behaviour Correspondent*

MUCH is now understood about the control of drinking in thirsty animals and feeding in hungry animals, but relatively little attempt has been made to understand how animals behave if they are made both hungry and thirsty and given access to both food and water. Problems of such motivational 'conflicts' are of great interest in finding out how behaviour in general is organised as most animals are probably in some form of conflict for much of their lives; for example, whether to attack a rival or flee, to feed or incubate eggs. McFarland and Lloyd (*Q. J. exp. Psychol.*, **25**, 48-61; 1973) now suggest that at least in the case of feeding-drinking conflicts some animals may resolve their conflicts by operating in a manner analogous to 'time of sharing' in computers.

It had previously been found (McFarland, *Rev. Comportement Anim.*, **4**, 64-73; 1970) that in doves, the normal course of feeding or drinking is characterised by pauses during which other types of behaviour occur. For example, a hungry dove would pause at various times during feeding and if water were present, it would drink during these pauses. Interestingly, the times when the pauses in feeding occurred were not affected by either the presence of external drinking stimuli or, within limits, on how thirsty the doves were. On this basis, the drinking was said to occur by 'disinhibition': it was allowed to occur during preprogrammed breaks in the dominant behaviour of feeding.

McFarland and Lloyd define a dominant behaviour as one which is always resumed following an interruption and a subdominant behaviour as one which is resumed only if the duration of the interruption is less than that of the preprogrammed break in an alternative (dominant) behaviour. Primarily hungry animals interrupted during feeding return to feeding, and primarily thirsty animals interrupted during drinking return to drinking, so that by suitable manipulation of food and water deprivation schedules, either feeding or drinking could be made to be the dominant motivation. Their new evidence suggests that not only does the dominant system determine when subdominant behaviours occur, it also determines how long the

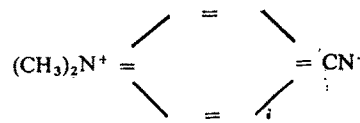
behaviour continues. They found that when a primarily hungry animal is interrupted while drinking or a primarily thirsty animal while feeding, the same behaviour is resumed after a short interruption, but changes to the alternative behaviour after a long interruption. In other words, if a subdominant behaviour is interrupted for a period which is longer than the pre-programmed break in the dominant behaviour, when the interruption is over it will be time for the animal to return to the dominant behaviour. An interruption which does not last longer than the allowable break in dominant behaviour will be followed by resumption of subdominant behaviour.

McFarland and Lloyd argue that when, as in the present case, the occurrence of an activity is determined by another motivational system both in terms of frequency and duration, then this should be called 'time sharing', by analogy with similar systems used on computers to overcome a similar problem—namely, to deal efficiently with simultaneous demands from several sources on limited parts of the machine.

Solvent reorganisation around a 'giant dipole'

from our *Chemical Physics Correspondent*

IT is difficult to find a new corner of science to develop these days, even a small corner can be elusive. This is, however, what Struve, Rentzepis and Jortner seem to have found in their paper on the kinetics of solvation (*J. chem. Phys.*, **59**, 5014; 1973). The thermodynamics of solvation of ions and molecules is a well studied subject but for kinetic information one must find a non-equilibrium, non-solvated species and then follow the development of solvation in the next few nanoseconds or less.



Jortner and his colleagues have solved this problem by studying the process after electronic excitation in *p*-dimethylaminobenzonitrile. In its ground state such a molecule is only weakly solvated, but its second singlet excited state corresponds to a quinoid formula (see figure) with its very large charge separation and a dipole moment of $77 \times 10^{-30} \text{ C m}$ (23 debye). Each end of the molecule will be heavily solvated, at least in polar solvents whose molecules will align themselves around the separated charges. At the instant of electronic excitation the molecule finds itself essentially unsolvated and it is the rate of subsequent solvation which

is of interest. The production of the species with non-equilibrium solvation is just the problem of exciting molecules to the quinoid state and this is accomplished with a laser flash at $37,600\text{ cm}^{-1}$ (265 nm) obtained as the fourth harmonic of a Nd^{3+} glass laser of duration about 5 ps.

The second half of the problem is to determine whether or not, at a subsequent moment of time, the quinoid state is yet solvated. This is answered by measuring the emission spectrum. If the solvation remains weak, as it will be in non-polar solvents such as methyl cyclohexane, the excited molecule loses its energy, first, by radiationless conversion to the lower excited singlet state and finally by fluorescence from this state at $28,600\text{ cm}^{-1}$ (350 nm). This process requires 20 ps and the fluorescence was detected photographically at the appropriate spectroscopic position. If the solvent consists of polar molecules, such as ethyl alcohol, the quinoid excited state is stabilised by solvation and is detectable as a new fluorescence emitter at $21,300\text{ cm}^{-1}$ (470 nm). Such fluorescence requires 50 ps to build up to maximum intensity and it is concluded that this delay corresponds to the time required for the polar solvent molecules to orient themselves around the giant dipole excited molecule. This seems a reasonable order of magnitude but the first experiments are not very precise; however, the experimental difficulties with delay paths, light shutters and so on are considerable. After all, in 50 ps light travels less than 2 cm.

New interpretation of Keweenawan rift

from our Geomagnetism Correspondent

THE 'midcontinent gravity high' of central North America is a linear belt of positive Bouguer anomalies which stretches about 1,300 km in a north-east-south-west direction from Lake Superior to Kansas. In numerical terms, the positive anomalies reach values as high as +60 mgal and for much of their length are flanked by parallel gravity lows reaching -100 mgal. According to Chase and Gilmer (*Earth Planet. Sci. Lett.*, **21**, 70; 1973), however, the shape of the belt may be more significant than the absolute values of gravity. The boundary between the highs and lows is sharply defined by very steep gravity gradients; and when the midcontinent gravity high is mapped by this boundary, it is seen to comprise several long linear segments offset from each other in a way reminiscent of the transform faults along oceanic ridges.

For this and other reasons Chase and Gilmer propose that the rocks produc-

ing the positive gravity anomalies may have resulted from plate tectonic processes which occurred during the Precambrian. That the rocks associated with the anomalies were formed during an episode of continental rifting is not, of course, a new idea in itself. In the Lake Superior region, for example, the positive anomalies lie over uplifted basaltic flows and intrusive gabbros of Keweenawan age, and the gravity lows are found over flanking basins containing younger Keweenawan sediments. The petrology and structure of the volcanics suggest that these rocks were formed during rifting of the Precambrian shield—an emplacement which zircon U-Pb ages show occurred over a short period between 1,140 and 1,120 million years ago. Moreover, evidence from gravity studies, magnetic anomaly data and boreholes indicates that the mid-continent gravity high and rifting are closely associated right through to Kansas.

But what Chase and Gilmer are suggesting is that the acknowledged rifting is the product of the sort of plate tectonic processes known to be occurring today—in other words, that the Keweenawan rift system is the result of the interaction of lithospheric plates during the late Precambrian. According to this view the offsets between the positive anomaly segments would correspond to transform faults and the segments themselves would correspond to oceanic spreading centres. The implication of this would be that the continental regions or blocks on either side of the rift would have acted as rigid plates during the rifting; and this in turn would place constraints on the geometry of the rift. Thus, assuming that these supposedly rigid plates have not been deformed subsequently, the present geometry of the rift should offer a test of the proposed rigidity.

The particular criteria to be applied in such a test are, first, that the supposed transform faults should lie along small circles about the same pole of relative rotation and, second, that the amount of rifting should increase with the sine of the angular distance from this pole. Using five supposed transform faults and the widths of the gravity high measured at eleven points, Chase and Gilmer show that a single pole of rotation may be defined to a remarkable degree of accuracy. The pole itself lies in northern New Mexico at 36°N , 107°W , the total rotation between the block to the north of the gravity high (called the Minnesota plate) and the block to the south (the Wisconsin plate) being 2.6° . At the 95% level the semi-major and semi-minor axes of the pole's oval of confidence are only 3.6° and 1.2° , respectively, and the limits on the rotation are $\pm 0.7^\circ$. The geometrical proper-

ties of the gravity high are thus indeed in close agreement with those to be expected from a plate tectonic model involving interacting rigid plates (apart from in the western Lake Superior region where matters are complicated by the presence of the mafic intrusions of the Duluth complex).

This discovery suggests, in turn, the need for a re-examination of the basic structure of the Keweenawan rift. Previous models of the structure have envisaged a basin 7–15 km deep, filled with dense Keweenawan lavas (supposedly causing the gravity high) and largely underlain by older continental crust. According to this view, the crustal separation would be represented by basaltic feeder dykes beneath the basin, and these would not be clearly defined by the observed gravity. The crustal separation involved in the hidden rifting would thus be difficult, if not impossible, to measure. This is why Chase and Gilmer used the width of the gravity high to represent the degree of rifting, realising that the two were not necessarily equal but assuming that the width of the lava-filled basin would at least be roughly proportional to the lesser width of the rifts hidden below.

But the fact that the width of the gravity high itself is in very close agreement with a plate tectonic geometry implies that this assumption is unnecessary and that the width of the gravity high is actually the width of the hidden rift. Chase and Gilmer thus go on to suggest that the lava basin did not form on a previously existing continental crust which had sunk but instead filled (and fills) the gap between the parted edges of continental plates. In this case it is no longer a matter, as previously thought, of having a relatively minor rift through which lava vented to give a lava basin controlled in width by the previous geology of the region. Instead, the Keweenawan is a major rift which ranges in width from more than 40 km at the southern end in Kansas to more than 85 km in central Lake Superior. A single gravity model is then sufficient to show that such a major rift is as consistent as the previously assumed structure with the observed gravity high.

Finally, from this particular example Chase and Gilmer go on to generalise in terms of an "evolutionary sequence" for continental rifting. They suggest that the initial stages of rifting may resemble the Rhinegraben or Baikal rift, that the next development may be similar to the East African Rift valleys, and that the Keweenawan rift type (the northern Red Sea being another and active example) represents the third stage. The final form would then be the familiar generation of new lithosphere at oceanic ridges as exemplified by today's southern Red Sea and the mid-Atlantic ridge.

Whys and wherefores of cosmic rays

How are cosmic-ray particles produced, and do some of them come from outside our Galaxy? These were the questions implicitly under discussion at a meeting held at the Royal Society on February 20 and 21.

On the second afternoon, T. Gold (Cornell University), V. L. Ginzburg (Lebedev Physical Institute, Moscow) and G. R. Burbidge (University of California, La Jolla) made their own distinctive contributions to the meeting. Gold put forward the case that pulsars could contribute at least electrons, protons and nuclei of mass around that of iron to the cosmic-ray beam. The cosmic radiation requires sources producing about 10^{34} J s⁻¹ and the average rate of production of energy by pulsars over a galactic lifetime of 10^{10} yr is reckoned to be 10^{36} J s⁻¹, so energetically the argument holds good. Gold suggested that the early and as yet undetected phases of pulsar evolution might well be instrumental in the formation of other parts of the cosmic-ray flux. He pointed out that there is already evidence from the distribution of pulsar periods, both by number and galactic latitude, that there is more than one way in which a pulsar can be created. The number distribution shows a marked bifurcation at pulsar periods of about 1 s, and Gold said that the probability of such a 'cut' in a single modal distribution is about 7%. The fact that pulsars with periods greater than 1 s are much less concentrated in the galactic plane than the others is added evidence of distinct forms of pulsar evolution.

A. W. Wolfendale (University of Durham) said earlier in the meeting that there is evidence of an upward bump in the differential energy spectrum of primary cosmic rays at energies of about 10^{15} eV, and this fitted nicely with Gold's contention that the energy spectrum of cosmic rays from pulsars should be fairly flat, with a cutoff at about that energy.

Ginzburg, whose paper was read in his absence, seems as convinced as he ever was that cosmic rays from outside the Galaxy are unlikely to contribute to the flux inside. He pointed out that if the intergalactic gas density were at the high end of the accepted range, 10^{-7} to 10^{-5} atoms cm⁻³, this would rule out some extragalactic models straight away. On the experimental side, he described several experiments which could have a crucial bearing on the problem. First, the measurement of the abundance of ¹⁰Be (half life 1.6×10^6 yr) would help differentiate between different galactic models. A complete lack of ¹⁰Be—indicating that it had all decayed—would

confirm that the halo of the Galaxy is involved in cosmic-ray propagation. Complete survival, on the other hand, would not necessarily rule out models involving the galactic disk as well as the halo. He described the measurement of the isotopic composition of cosmic rays as "on the horizon". Other experiments put forward by Ginzburg included measuring the energy spectrum of relativistic positrons, essentially to find a 'characteristic time' for cosmic rays in the Galaxy and monitoring the γ -ray flux from the galactic centre and from the Magellanic Clouds. The latter would require γ -ray detectors some 300 times more sensitive than those at present available but would place an upper limit on the input of cosmic rays from outside the Galaxy.

Following Ginzburg's advocacy of galactic models, Burbidge put forward the case for thinking that some, though not all, cosmic rays found in the Galaxy

are produced outside its confines. What of the energy implications? Burbidge recalled that filling the whole Universe with cosmic rays at the density found near the Earth (10^{-5} J cm⁻³) would require every galaxy to produce 10^{56} J in a lifetime of 10^{10} yr—1% of its rest mass, for some people a worryingly high figure. If, however, calculations are based on the assumption that only galactic clusters and superclusters (1% of the volume of the Universe) are filled with cosmic rays then only 10^{-4} of the rest mass is involved.

Arguing against the objection that extragalactic cosmic rays could not get into the Galaxy, Burbidge said that particles do diffuse into the outer parts of radio galaxies such as Cen A, so that it is difficult to see how particles will fail to get to the Earth. Burbidge appealed to "nature and more theoretical studies", which have up to now tended to be confined to galactic models.

Earthquake history

SEISMOLOGISTS and others interested in earthquake phenomena will probably already know of the two volume *Earthquake History of the United States*, originally published in 1928 and since updated several times. The latest revision brings the data together in one volume, published by the US Department of Commerce (National Oceanic and Atmospheric Administration, 1973).

The data now include earthquakes from the mid-seventeenth century (for the eastern states at least) up to 1970; as well as the catalogue of dates, intensities and places, earthquakes are given short 'eye witness' descriptions as their intensity and destructiveness justify, and maps indicate the 'seismic risk' in different parts of the contiguous United States. Anybody interested in older events (outside of the United States, of course) is referred to Milne's *A Catalogue of Destructive Earthquakes AD7 to AD 1899* (Br. Ass. Advmt. Sci., 649; 1911).



Part of 130 acres of land devastated by 1964 Good Friday shock in Turnagain Heights area of Anchorage, Alaska.

Reality of bubble nuclei

from our Nuclear Theory Correspondent

MANY studies of nuclei by electron and nucleon scattering have shown that some are spherical whereas others are more or less strongly deformed into spheroidal or ellipsoidal shapes. Studies of their excited states show that the deformed nuclei have characteristic rotational spectra but that the spherical nuclei can often be excited into a series of vibrational modes.

Wheeler pointed out long ago that these are not the only conceivable nuclear shapes. One can imagine not only wobbly drops of various shapes but more exotic configurations like bubbles and toroids. Is it possible, for example, to form a bubble of nuclear matter and if so would it be stable?

It is plainly possible to imagine a nucleus from which all the nucleons in the lowest *1s* state have been suddenly removed by a direct interaction, leaving the other nucleons undisturbed. This would be a highly excited nucleus but it might live long enough to qualify as a bubble nucleus. It is also conceivable that bubble nuclei are sometimes formed in the turbulent nuclear motions resulting from very energetic collisions of heavy ions. These bubble nuclei are likely to be highly excited but it is also possible that bubble configurations are found at low excitations or even in the ground state.

Whereas heavy nuclei can usefully be thought of for some purposes as a drop of fluid of almost uniform density, light nuclei have density distributions that can be interpreted as α -like clusters in regular array. Thus ^{12}C has a density distribution similar to that of three α s at the vertices of an equilateral triangle and ^{16}O as four at the vertices of a tetrahedron. Such nuclei may have a low central density and may be classed as bubble nuclei.

Calculations of the stability of bubble nuclei were made by Siemens and Bethe (*Phys. Rev. Lett.*, **18**, 704; 1967) using the semi-empirical mass formula and they found that some large spherical bubble nuclei may be stable against symmetry-preserving breathing expansions and contractions, but they did not investigate their stability against other distortions.

The stability of bubble-shaped nuclei against symmetry-preserving breathing deformations has also been investigated by Krishnan and Pu (*Phys. Lett.*, **47B**, 225; 1973) using the energy density formalism. They find that bubble nuclei are stable only for very large mass numbers ($A \simeq 700$), far beyond the region known at present. Lighter nuclei do not have stable bubbles.

A series of calculations using the liquid

drop model shows that bubble nuclei are unstable (Wong, *Ann. Phys.*, **77**, 279; 1973) and this confirms other calculations that do not take the shell structure of nuclei into account. If, however, the shell structure is included, some bubble nuclei with a low density of single-particle states near the Fermi surface are found to be stable. These calculations are made by assuming that the nuclear potential follows the density distribution; the energies of the single-particle states can then be found by standard procedures. The reduced potential near the centre of a bubble nucleus raises the energies of those single-particle states whose wave functions overlap with this region, and these are states of low angular momentum. Thus in a bubble configuration the energies of states of low angular momentum are raised relative to those of states with high angular momentum and this gives a new series of magic nucleon numbers for pronounced bubble configurations.

In this way Wong (*Phys. Lett.*, **41B**, 451; 1972) found the new magic numbers 18, 34, 50, 70, 80, 104, 120... at various deformations. Use of the Strutinsky method to evaluate the shell corrections then indicates ^{36}Ar , ^{84}Se , ^{138}Ce and ^{200}Hg as possible bubble nuclei in their ground states.

More detailed calculations may be made with the Hartree-Fock theory using a realistic effective nucleon interaction and some results of Curry and Sprung (*Nucl. Phys.*, **A216**, 125; 1973) for ^{36}Ar are shown in Fig. 1. Also shown is the corresponding distribution for ^{40}Ca less that of an α particle. Allowing for

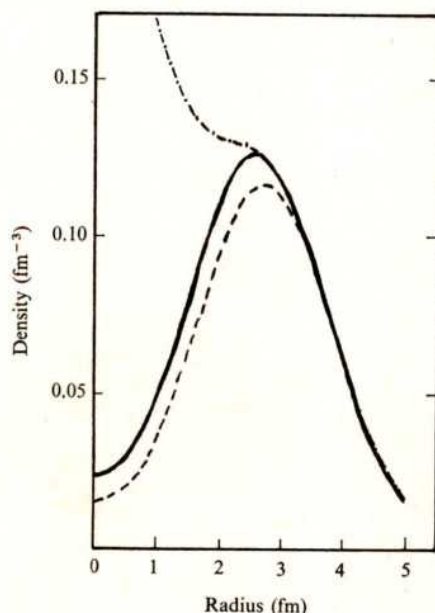


Fig. 1 Density distribution of ^{36}Ar (—) compared with that of ^{40}Ca (— • —) and the density difference of ^{40}Ca and an α particle (---) obtained by Hartree-Fock calculations. The central bubble is well marked, and is identified as an α hole (Curry and Sprung).

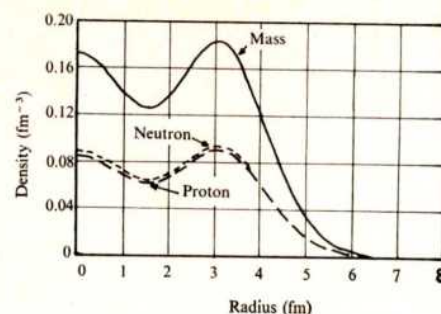


Fig. 2 Density distribution of ^{68}Se showing the region of minimum density centred on a spherical shell of radius about 1.5 fm (Davies, Krieger and Wong).

a slight inward relaxation due to self-consistency effects the two distributions correspond very well, suggesting that ^{36}Ar has a bubble configuration corresponding to ^{40}Ca with an α particle removed from its centre. These nucleons in the α particle are not, however, in *s* states; removal of *s*-state nucleons would give ^{36}Ar in a highly excited state and a density distribution quite different from that shown.

Further calculations for a series of possible bubble nuclei have been made by Davies, Krieger and Wong (*Nucl. Phys.*, **A216**, 250; 1973) and they confirm the bubble nature of a low energy configuration of ^{36}Ar and in addition find marked bubble effects in ^{200}Hg and ^{116}Ce and ^{138}Ce . A different type of bubble is found in ^{68}Se ; the region of lower density is centred on a spherical shell of radius about 1.5 fm instead of the origin (see Fig. 2).

Detailed examination of the results of these calculations shows that in these nuclei the bubble configurations are preferred because they reduce the density of single-particle states in the region of the Fermi surface. The expected raising of states of low angular momentum relative to those of high angular momentum is indeed found in some bubble nuclei. With the same model, the strength of the spin-orbit potential of the Thomas form (proportional to the radial derivative of the density distribution) depends on the relative amplitudes for finding the nucleon at the surface or near the centre of the nucleus. This implies that for bubble nuclei the spin-orbit doublets may be inverted and the likelihood of this is greater for smaller values of orbital angular momentum. This effect is also observed in some nuclei.

These investigations show that the usual picture of nuclei having an almost uniform central density is sometimes far from the truth. There are several nuclei that show pronounced minima or bubbles in their centres and others have even more complicated departures from uniform density.

Evidence for a ~ 4.5 aeon age of plagioclase clasts in a lunar highland breccia

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Argon from neutron-irradiated mineral separates and whole rock samples of a metamorphosed breccia (65015) from Apollo 16 has been analysed with a large number of gas extraction steps in order to obtain a high resolution in the apparent ages and to identify the gas released from different sources. The results on plagioclase show a ^{40}Ar - ^{39}Ar plateau age of 3.98×10^9 yr, which we attribute to the time of metamorphism, and an age of $\sim 4.5 \times 10^9$ yr in the high temperature fraction. Correlation of the release pattern with ^{37}Ar instead of ^{39}Ar permits the association of the $\sim 4.5 \times 10^9$ yr age with relict plagioclase which were demonstrated in previous petrographic and Rb-Sr studies as being unequilibrated. This result suggests that it is possible to identify lithic components which represent the early lunar crust.

THIS investigation attempts to decipher the premetamorphic age of the protolith of a recrystallised breccia from Apollo 16 using the ^{40}Ar - ^{39}Ar technique. Petrographic studies have revealed in some lunar breccias, clasts which have not been brought into chemical equilibrium with the recrystallized groundmass¹. Detailed Rb-Sr studies have shown that some plagioclase clasts were not isotopically equilibrated with the matrix and consequently show isotopic evidence of a pre-metamorphic history^{2,3}. The study of such unequilibrated rocks is of major importance in penetrating the time barrier at $\sim 4.0 \times 10^9$ yr, reflecting the terminal lunar cataclysm³⁻⁶. Such results are directly applicable to the characterization of the early lunar crust and the time scale for its evolution.

Rock 65015 was known to contain Sr isotopic relicts in plagioclase mineral separates⁴ and was therefore selected for a comprehensive study with the ^{40}Ar - ^{39}Ar technique⁷. An optimal experiment would be an analysis of macroscopically recognisable plagioclase clasts which were known to be almost totally unequilibrated. Such a sample of 0.5 mg weight has provided the nearest 65015 data point to the primitive Sr isotopic composition of BABI (see Fig. 1 of Tera *et al.*⁴). A similar ^{40}Ar - ^{39}Ar analysis unfortunately requires much more material than is currently available. As a compromise, a mineral separate was chosen which was relatively large in size (47 mg) but which was still known to be distinctly off the internal Rb/Sr isochron by a small amount. This sample (plag B) may be seen in fig. 4 of Papanastassiou and Wasserburg² and fig. 1 of Tera, *et al.*⁴ Although it may superficially appear that this sample lies on the internal isochron, it must be recognised that the analytical errors are the size of the central dots. Further, the five different plagioclase separates lie precisely on a straight line which is quite different from the internal isochron, and the position of plag B along this line indicates that about 14% of plag B is composed of unequilibrated clasts as distinct from groundmass plagioclase. This was supported by inspection of the mineral separate B, which showed a higher ratio of large ($\sim 100 \mu\text{m}$) plagioclase cleavages to finer grained groundmass plagioclase

than found in another plagioclase separate, C, which is also closer to the internal isochron. Plag C is itself off the isochron by several times the possible errors, which originally led to the search for the effects in B and subsequently in the more extreme examples of non-equilibration as found in the macroscopically identifiable clasts in the uncrushed rock.

Because of complexities in both the technique (compare ref. 6) and in the breccia itself, Ar analyses were made on a whole sample and several mineral separates of 65015 using a large number of incremental heating steps in order to provide a high resolution in variations of $^{40}\text{Ar}/^{39}\text{Ar}$. It was anticipated that such high resolution measurements, together with other isotopic and petrographic observations, would allow an isolation of the appropriate parts of the 'age spectra' associated with relict retentive phases. To obtain a sharper distinction between the Ar released at low and high temperatures, presumably reflecting the outgassing from lattice sites with different retentivities, we carried out 30-50 degassing steps between $\sim 300^\circ\text{C}$ and $1,500^\circ\text{C}$ on each sample, some of which were repeated extractions at the same temperature. All samples were part of the same neutron irradiation, which minimised comparative errors. The amounts of ^{40}Ar from the radioactive decay of ^{40}K and the amounts of ^{39}Ar produced by neutron capture on ^{39}K were determined after appropriate corrections. (Ar data were corrected sequentially for: spectrometer discrimination, decay of ^{37}Ar ($\lambda_{37} = 0.01975 \text{ d}^{-1}$) and ^{39}Ar ($\lambda_{39} = 7.2 \times 10^{-6} \text{ d}^{-1}$) before analysis; Ar interferences from neutrons on Ca and K; normalisation to standard neutron fluence; subtraction of procedural blank with assumed air composition in units of $10^{-9} \text{ cm}^3 \text{ STP } ^{40}\text{Ar}$ of $(1.5 \pm 0.5) - (2.0 \pm 0.7)$ at $<1250^\circ\text{C}$, (3 ± 1.2) at 1300°C , (7 ± 3) at $1,400^\circ\text{C}$, (17 ± 8) at $1,500^\circ\text{C}$. We used: $(^{36}\text{Ar}/^{37}\text{Ar})_{\text{ca}} = 0.000305$, $(^{39}\text{Ar}/^{37}\text{Ar})_{\text{ca}} = 0.000732$, $(^{38}\text{Ar}/^{39}\text{Ar})_{\text{K}} = 0.01$, $(^{40}\text{Ar}/^{39}\text{Ar})_{\text{K}} = 0.01$.) The apparent ages were calculated from the $^{40}\text{Ar}/^{39}\text{Ar}$ ratios for each temperature release and are shown in Fig. 1A for the whole rock and two mineral separates. (Conversion factors for LAV3 irradiation: $C_{39}(\text{K}) = 8.02 \times 10^{-4} \text{ cm}^3 \text{ STP } ^{39}\text{Ar}/\text{gK}$, $\text{K}/\text{Ca} = (0.54 \pm 0.02) ^{39}\text{Ar}/^{37}\text{Ar}$. Constants in age calculations: $\lambda_{39} = 4.27 \times 10^{-10} \text{ yr}^{-1}$, $\lambda_{40} = 0.585 \times 10^{-10} \text{ yr}^{-1}$, $^{40}\text{K}/\text{K} = 1.19 \times 10^{-4}$.) The age scale is enlarged to make the fine structure visible.

The apparent ages of all samples, including two which are not illustrated, increase very rapidly to a local maximum at $\sim 600^\circ\text{C}$, followed by a decrease of $(0.05-0.1) \times 10^9$ yr. The apparent ages of the plagioclase and whole rock recover above about 840°C , whereas those of the pyroxene remain almost constant up to 900°C . A normal experiment with less resolution would not show any of this fine structure, but would give very well defined plateaux of $(3.90-4.05) \times 10^9$ yr for all samples between $500-900^\circ\text{C}$. Above 900°C , the ages of both pyroxene and whole rock decrease by $\sim 0.8 \times 10^9$ yr and $\sim 0.3 \times 10^9$ yr, respectively, whereas plagioclase exhibits a rapid increase in apparent age. The highest age of 4.5×10^9 yr is approached in a regular sequence of precise data which, except for the very last point, are not affected by blank corrections (Fig. 1B). Because the total rock is a polymineralic aggregate, its complex behaviour is not un-

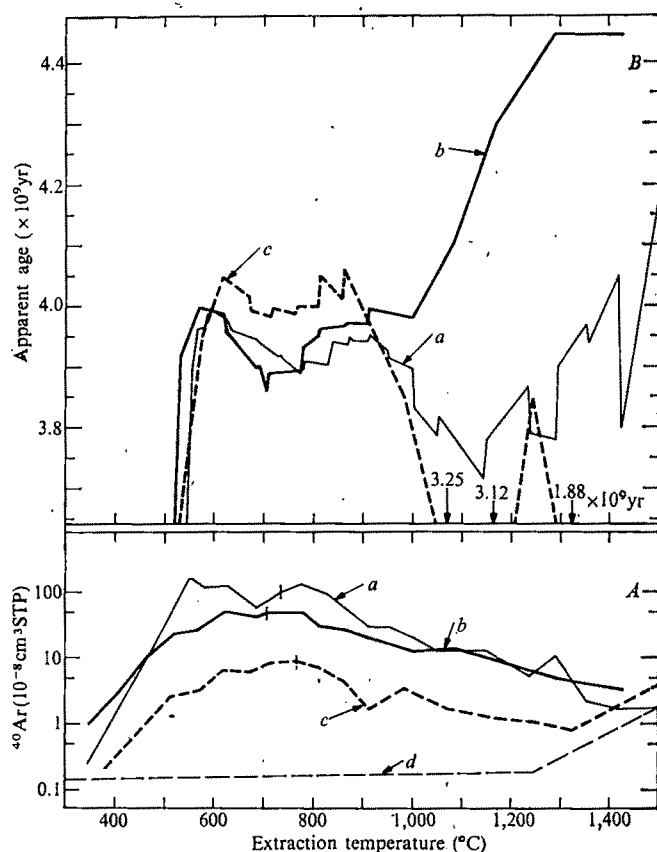


FIG. 1 Differential ^{40}Ar release (A) and apparent ages (B) against extraction temperature for: a, total rock sample (64 mg); b, plagioclase B (47 mg); c, pyroxene (57 mg) separates of Apollo 16 breccia 65015; d, blank. A, The amounts of ^{40}Ar released in multiple extractions at the same temperature have been added. Vertical bars mark the temperature where $\sim 50\%$ of the total ^{40}Ar has been degassed and illustrate that the low temperature release of all samples is dominated by a high K phase. In the plagioclase curve at $\sim 1,000^\circ\text{C}$ there is a hint of a structure possibly due to the release from a high temperature 'phase'. The average blank amounts, determined from 35 procedural blank runs with maximum variations of 50% from the given curve, affect only the last extraction of each sample, but clearly not the $1,290^\circ\text{C}$ plagioclase datum. B, To separate visually the apparent age points from multiple extraction steps at constant temperature T , the age of the n th step is plotted at $(T + 5n)^\circ\text{C}$ on an expanded age scale covering the significant range. The low temperature apparent age patterns are characterised by a similar behaviour for all samples, which we attribute again to an ubiquitous high K and relatively unretentive mineral phase. Above $\sim 900^\circ\text{C}$ pyroxene shows its characteristic decrease, which is not understood. Plagioclase, on the other hand, after a well documented plateau ($865\text{--}1,000^\circ\text{C}$) increases rapidly to 4.46×10^9 yr at $1,290^\circ\text{C}$. The whole rock age pattern is a complex synthesis of all phases.

expected at this resolution. The high temperature decrease in the age of the whole rock sample can be attributed to the inclusion of pyroxene, for which the decrease at high temperatures is here again established as characteristic (see ref. 8), although it is not yet understood. The maximum ages at 600°C differ by up to 0.05×10^9 yr, but the similarity in the low temperature behaviour of all samples suggests a source for this characteristic pattern in a ubiquitous accessory phase which is not readily removed by mineral separation techniques on such fine grained material. Albee *et al.*¹ have noted that 90% of the potassium in 65015 resides in non-stoichiometric, irregularly dispersed, very fine patches, which appear to be devitrified glass (quintessence). This phase contributes a large fraction of the total ^{40}Ar released at low temperatures (Fig. 1B), as demonstrated by the fact that at low temperatures K/Ca is 10–100 times larger than at high temperatures (Fig. 2C).

In summary, we conclude that by usual standards, the $^{40}\text{Ar}/^{39}\text{Ar}$ ratios in the region between 550 to 900°C define reasonable plateaux corresponding to ages between 3.90×10^9 yr and 4.05×10^9 yr. The complex age spectra, however, are apparently the result of a blend of a common K-rich phase with the major minerals plagioclase and pyroxene. It is evident from these refined age patterns that a variety of processes must be taking place during the differential thermal analyses. The causes for the complex age spectra are not well established. A reasonable mechanism to explain the characteristic age peak at $\sim 600^\circ\text{C}$ is redistribution of ^{39}Ar because of recoil from fine grained quintessence. Typically, the low initial ages would be interpreted as a result of ^{40}Ar loss on the lunar surface. Because of the low temperature fine structure, and the possibility of ^{39}Ar redistribution, the exact extent of any ^{40}Ar loss on the lunar surface is uncertain.

We will now focus on the plagioclase results and the study of Ar released from plagioclase in the dominating presence of Ar released from other phases. It is our purpose to identify the plagioclase ages and separate them from the ages of high K impurities. Traditionally, the apparent ^{40}Ar – ^{39}Ar ages are plotted against the cumulative fractional ^{39}Ar release (Fig. 2A). In polymineralic systems containing minerals with very different K concentrations, the age pattern in this representation may be dominated by rare high K phases, which have a preponderance of total K in the sample.

Evidence for this is seen both in the fact that the total K in plagioclase separate B inferred from total ^{39}Ar is 1,475 p.p.m., in contrast with ~ 500 p.p.m. for plagioclase in 65015 determined by electron microprobe¹, and in the fact that the dominant ^{39}Ar and ^{40}Ar release occurs at low temperatures (Figs 1A and 2A). As plagioclase B is $\sim 99\%$ pure, this would require that the additional phase which releases argon at low temperatures can only amount to about one percent of the sample weight but must have a very high K content. An admixture of only 1.5% of quintessence with 6.4% K (ref. 1), can account for two-thirds of the K in plagioclase B.

In contrast to the potassium, Ca in the plagioclase is $\sim 13\%$ as compared to only $\sim 2\%$ in quintessence¹. Therefore ^{37}Ar release from our sample is related entirely to the release from plagioclase and not the K-rich, low Ca impurities. A true plagioclase plateau should be associated with the ^{37}Ar released. Figure 2B shows the data in this representation. There is a distinct low temperature peak in the high K/Ca regime (fig. 2C) and a reasonably well defined plateau over the temperature region $865\text{--}1,000^\circ\text{C}$, containing 35% of the ^{37}Ar and corresponding to an almost constant K/Ca ratio of 0.005, which is appropriate to plagioclase. It now becomes evident that the $\sim 600^\circ\text{C}$ peak is a disturbance. Since it covers approximately the first 50% of the ^{39}Ar release (Fig. 2A), it would normally be interpreted as an intrinsic part of the plateau, but it is identified in the ^{37}Ar plot as a minor contribution from a high K phase in the age spectrum of plagioclase. In the high temperature regime, the sharp increase of the apparent age to 4.5×10^9 yr is

TABLE 1 Argon ages in breccia 65015 (inferred from plagioclase mineral separate B)

	Gas retention age ($\times 10^9$ yr)*	Exposure age (m.y.)†
865–1,000° C		
'equilibrated plagioclase'	3.98 ± 0.01	466 ± 1
1,290° C		
'unequilibrated plagioclase clasts'	4.46 ± 0.05	≥ 505

* When comparing these ages with those from other laboratories or other irradiations, an additional error of $\pm 0.04 \times 10^9$ yr from the uncertainty in C_{39} (K) has to be included.

† Calculated with production rate $P_{38} = 1.4 \times 10^{-8}$ cm³ STP $^{38}\text{Ar}/\text{g Ca my}$ and excluding an error of ± 17 m.y. from C_{37} (Ca).

more obvious and is correlated with the last 15% of the ^{37}Ar release, as compared with the last 5% of the ^{39}Ar release. It should be noted that these observations depend critically

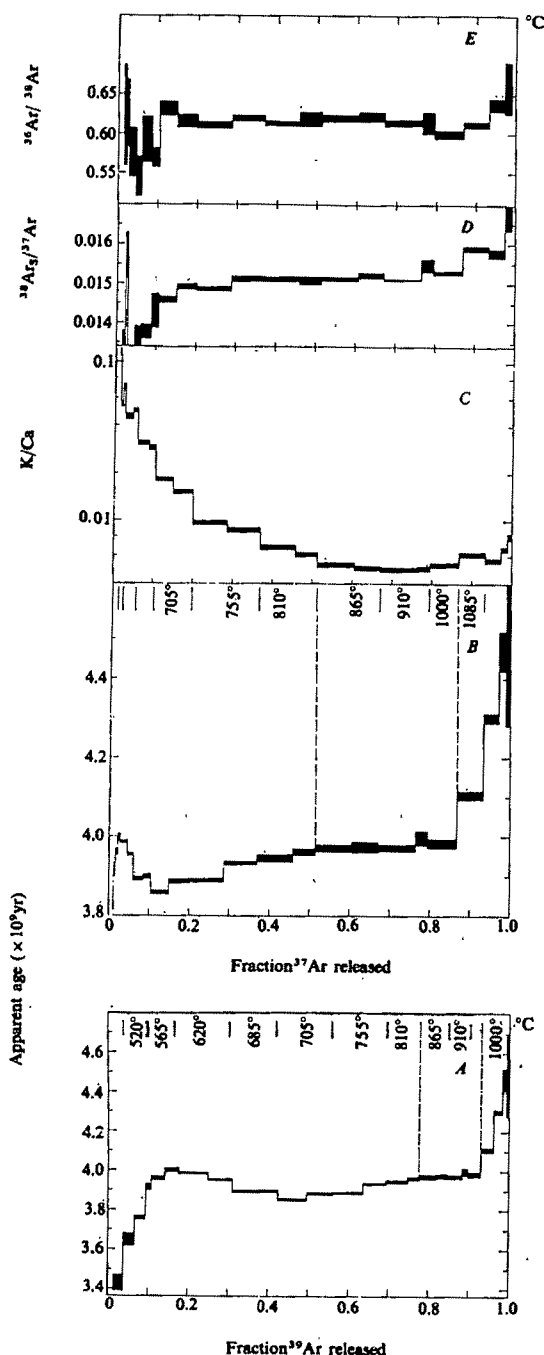


FIG. 2 Isotopic composition of Ar from a plagioclase separate of Apollo 16 breccia 65015, against (A) fractional release of ^{39}Ar , and (B-E) fractional release of ^{37}Ar . The graph of apparent age against ^{39}Ar (A) is dominated by Ar released from K-rich, Ca-poor accessory phases. Graphs against ^{37}Ar emphasise the composition of Ar from Ca-rich plagioclase and reveal (between vertical dashed lines) a well-defined intermediate plateau at 3.98×10^9 yr (B). This is associated with the appropriate K/Ca for plagioclase (C), a constant $^{36}\text{Ar}/^{38}\text{Ar}$ (E) at a value appropriate for spallogenic Ar, and a well defined plateau in $^{36}\text{Ar}/^{37}\text{Ar}$ (D) indicating a cosmic-ray exposure age of 466 m.y., all ascribed to plagioclase degassed or recrystallised by metamorphism at 3.98×10^9 yr. The last 10% of ^{37}Ar release shows increases in apparent age associated with increases in K/Ca and $^{36}\text{Ar}/^{37}\text{Ar}$, but constant $^{36}\text{Ar}/^{38}\text{Ar}$. We attribute this to unequilibrated and incompletely degassed plagioclase clasts from the protolith of 65015 previously recognised by petrographic and Rb/Sr studies, and infer for them a formation age of $\sim 4.5 \times 10^9$ yr, and exposure to cosmic rays before 3.98×10^9 yr.

on high resolution data. If there were only ten equal steps, then the last four releases would have given an apparent age of 4.2×10^9 yr.

It is most plausible to associate the plateau of 3.98×10^9 yr on the ^{37}Ar plot with plagioclase which has completely lost ^{40}Ar in a metamorphism at that time, and to further associate the age peak at $\sim 600^\circ\text{C}$ with the crystallisation of quintessence at approximately the same time. The age of 4.5×10^9 yr obtained in the highest temperature release from the plagioclase separate is one of the most interesting and possibly most important results of the present work. It is plausible to correlate this age with the plagioclase clasts which have not been thoroughly equilibrated and outgassed during this metamorphism. It is conceivable, however, that the high age values are artefacts. As a test of this, the $^{36}\text{Ar}/^{38}\text{Ar}$ and $^{36}\text{Ar}/^{37}\text{Ar}$ ratios can be inspected for irregularities (see Figs. 2D and E). Again the ^{37}Ar plot is the most convenient representation, since Ca is the predominant target nucleus for the production of spallogenic Ar by cosmic rays. It can be seen that the $^{36}\text{Ar}/^{38}\text{Ar}$ ratio is remarkably constant at 0.62, in good agreement with typical spallation values on Ca and Fe (ref. 9). There is a hint of slight fluctuations ($\sim 6\%$) in the last 10% of the ^{37}Ar release. The $^{36}\text{Ar}/^{37}\text{Ar}$ shows a broad plateau after the first 10% and up to the last 15% of ^{37}Ar release. The pattern of $^{36}\text{Ar}/^{37}\text{Ar}$ characterises a well defined cosmic-ray irradiation age, with spallation $^{36}\text{Ar}_s$ exactly correlated with the Ca target, as measured by ^{37}Ar . The last $\sim 15\%$ of the ^{37}Ar release shows a small but distinct ($\sim 10\%$) increase in $^{36}\text{Ar}_s/^{37}\text{Ar}$ correlating with the high $^{40}\text{Ar}/^{39}\text{Ar}$ part of the age spectrum and also some variations in K/Ca. The most obvious interpretation is to consider this as reflecting a pre-irradiation of the older plagioclase clasts before the formation of the recrystallised breccia, in which case the clasts must have resided for some time in the upper few metres of the lunar surface before the last major collision causing the metamorphism. The increase in $^{36}\text{Ar}_s/^{37}\text{Ar}$ cannot be due to a marked change in chemistry (for example, Fe or Ti enrichment¹). A production of ^{38}Ar by neutron reactions on ^{37}Cl is highly improbable since $^{36}\text{Ar}/^{38}\text{Ar}$ is constant. If the $^{36}\text{Ar}_s/^{37}\text{Ar}$ increase is an artefact, it is a factor of two smaller than that manifested in the $^{40}\text{Ar}/^{39}\text{Ar}$ ratio, and a factor of $\sim 10^2$ smaller in the number of ^{40}Ar atoms as compared with the $^{36}\text{Ar}_s$. Thus we do not consider the change at high temperatures in the ratios of the spallogenic isotopes to be indicative of artefacts in $^{40}\text{Ar}/^{39}\text{Ar}$ ratios. The average K/Ca is 20% higher in the plagioclase which apparently retains Ar from dates before 3.98×10^9 yr. Observations on the cores of plagioclase clasts show that they are usually slightly lower in K than the rims and matrix plagioclase, although some do have relatively higher K contents.

The correlation of gas release from plagioclase with the ^{37}Ar release enables us to estimate that $\sim 15\%$ of plagioclase B can be related to high ages, which is in striking agreement with $\sim 14\%$ inferred from Rb/Sr studies of the same separate. This provides another very important and independent argument for the association of the $\sim 4.5 \times 10^9$ yr ages with unequilibrated plagioclase clasts. The argument is considerably strengthened by the observation of a similar $^{40}\text{Ar}/^{39}\text{Ar}$ increase to $\sim 4.1 \times 10^9$ yr in approximately the last 5% of the ^{37}Ar release from plagioclase C, which Rb/Sr studies indicate contains only $\sim 8\%$ unequilibrated clasts.

Thus, the analysis of the high resolution differential thermal release patterns of all Ar isotopes from neutron irradiated samples of the lunar rock 65015 indicates that this breccia was metamorphosed and recrystallised 3.98×10^9 yr ago, at about the time of the terminal lunar cataclysm. Part of the plagioclase shows a distinctive high temperature age maximum of 4.46×10^9 yr which is interpreted as being due to incomplete outgassing of old plagioclase clasts

during the metamorphic event. These results are in support of the reports by other workers^{6,10} on evidence for more ancient ages in some lunar samples. In the instance of 65015, the ages gain credence from corroborating petrographic and Sr isotopic studies, but the firm assignment of a precise time to some part of a ^{40}Ar - ^{39}Ar release pattern requires a complete understanding of the nature of ^{40}Ar - ^{39}Ar variations. The plagioclase clasts are tentatively considered to have original ages of formation of at least 4.46×10^9 yr and therefore provide evidence for the existence of some early Lunar crust with plagioclase of this composition formed 4.5×10^9 yr ago. There is no evidence in the data for intermediate metamorphic events. The data are consistent with a simple history of formation of some constituents of the breccia before $\sim 4.5 \times 10^9$ yr and subsequent formation of the breccia followed by severe metamorphism 3.98×10^9 ago. This is in agreement with a two stage evolution model for the lunar crust with these identical age limits established by Pb/U, Th systematics for an extensive suite of samples which includes 65015.

The high ^{40}Ar - ^{39}Ar age of part of the plagioclase is accompanied by a slightly older exposure age relative to the rest of the breccia, which is indicative of a pre-irradiation. Generalising the 65015 results, we propose that the protolith of many impact breccias includes in part material which resided for some time in the outer few meters of the lunar surface before the metamorphism which resulted from major impacts $\sim 4.0 \times 10^9$ yr ago.

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Evidence for continental crust beneath the Faeroe Islands

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Recent crustal seismic refraction experiments in the North Atlantic indicate that the Faeroe Islands are underlain by continental crust. This suggests that the whole Rockall-Faeroe Plateau may be a microcontinent which formed during the early evolution of the North Atlantic.

THE Faeroe Islands lie at the north-eastern end of a region of shallow banks in the North Atlantic known as the Rockall-Faeroe Plateau (Fig. 1), which is separated from the United Kingdom continental shelf by the Rockall Trough and Shetland-Faeroe Channel. It has recently been demonstrated that Rockall Plateau at the south-western end of this region is underlain by ancient continental crust¹⁻³. The Faeroe Islands also lie at the south-eastern end of another shallow feature known as the Iceland-Faeroe Ridge, but this is fundamentally different from Rockall Plateau in that it is underlain by anomalous Icelandic-type oceanic crust formed by differentiation from the mantle over the last 60 m.y., during the evolution of the North Atlantic by seafloor spreading^{4,5}. The

Faeroe Islands themselves are formed by a thick pile of nearly flat-lying lavas of early Tertiary age whose base is not seen⁶; the exposed lavas are radiometrically dated⁷ at 50 to 60 m.y. which makes them contemporaneous with the early Tertiary volcanic activity of north Britain and Greenland. The nature of the crust beneath the Faeroes is thus problematical—is it continental or anomalous oceanic? The answer is crucial to our understanding of the Tertiary development of the north-eastern North Atlantic. We give new evidence from a crustal seismic refraction experiment which indicates that the lavas of the Faeroe Islands are underlain by continental crust about 30 km thick.

Before the present work, two items of evidence indicated that the Faeroe Islands may be continental. First, Bott and Watts⁸ showed, in making a pre-Tertiary continental reconstruction of the north-eastern North Atlantic, that if Rockall Plateau is regarded as continental then the Faeroe Block must also be continental to give an acceptable fit with Greenland. Second, a steep Bouguer anomaly gradient occurs between the Iceland-Faeroe Ridge and the Faeroe Block, indicating that the mean crustal density is significantly less beneath the block than the ridge and suggesting funda-

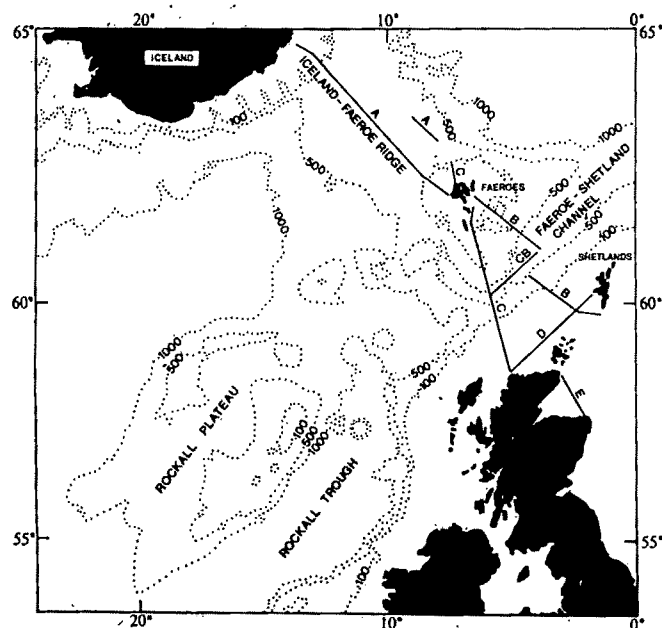


FIG. 1 Map of the region between Scotland and Iceland, showing the position of the Faeroe Islands in relation to Rockall Plateau and the Iceland-Faeroe Ridge, and the lines of shots fired during the North Atlantic Seismic Project. The following bathymetric contours are shown: 100, 500 and 1,000 fathoms.

mentally different types of crust beneath the two adjacent regions^{4,5}. Conversely, an early seismic refraction experiment made by Pálmason⁹ gave a 6.4 km s^{-1} basement beneath 2.5–4.5 km of lavas, indicating a structure similar to that beneath Iceland¹⁰. Another seismic experiment made by Casten¹¹, however, gave an unreversed basement velocity of 5.9 km s^{-1} which would be in accord with normal continental crust.

During a major seismic refraction project between Scotland and Iceland in 1972, geophex shots of 300 to 2,000 pound were fired along the lines A to E shown in Fig. 1. Recording stations were situated on Iceland, Faeroe Islands, Shetland and Orkney Islands; and the Scottish mainland. Shots were also recorded at sea by MV *Miranda* and the Russian ship *Michail Lomonosov*. Seven recording stations were placed on the Faeroe Islands, six provided by Aarhus University (in cooperation with Hamburg and Kiel Universities) and one by Durham (Fig. 2). A preliminary interpretation of that

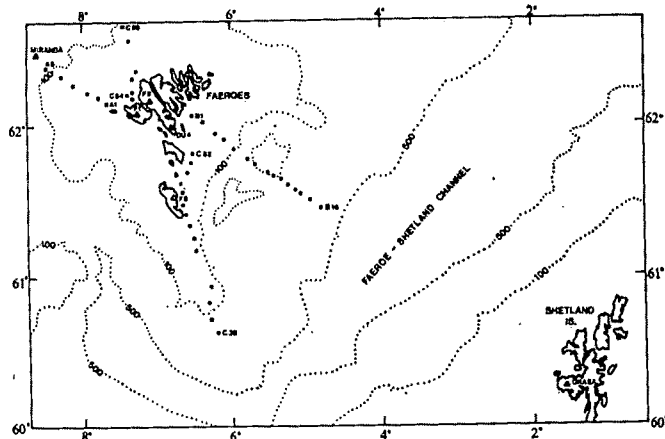


FIG. 2 Map showing the shot positions (●) and seismic recording localities (triangles) used in the present analysis. 100 and 500 fathom bathymetric contours are shown. Shots B6, C40, C53 and C57 were misfires and are not shown.

part of the project which concerns the crustal structure of the Faeroe Block is given in this paper, using data received at four of the Faeroe stations, the ship *Miranda* and the United Kingdom Atomic Energy Authority (UKAEA) array station on the Shetland Islands (Fig. 2).

The P_g arrivals from shots on the Faeroe shelf fall into two groups as follows: (1) those from shots on the north Faeroe shelf, and (2) those from shots to the east and south of the Faeroe Islands. These two groups need to be treated separately. The records from shots on the north Faeroe shelf yield apparent velocities as follows: line A: DU4– $6.17 \pm 0.14 \text{ km s}^{-1}$, F1– $5.98 \pm 0.08 \text{ km s}^{-1}$, F2– $6.18 \pm 0.12 \text{ km s}^{-1}$, *Miranda*– $5.98 \pm 0.22 \text{ km s}^{-1}$; line C (north-west): DU4– $5.92 \pm 0.08 \text{ km s}^{-1}$. The observations at station F1 and on *Miranda* roughly reverse line A suggesting a true P_g velocity of 5.95 km s^{-1} between these stations. A time-term analysis¹² on all the north Faeroe shelf data available, including that of Pálmason⁹, yields a value of P_g velocity of $6.18 \pm 0.09 \text{ km s}^{-1}$. We have looked again at Pálmason's earlier results⁹, and we find that if first arrivals only are used then the velocity of the basement beneath his reversed line is 5.9 km s^{-1} . Thus our results suggest that the true P_g velocity in this northern region is within the range 5.9 to 6.2 km s^{-1} . This value is significantly lower than the velocity of 6.4 km s^{-1} for the lower crustal layer beneath Iceland but it is fairly typical of metamorphic basement rocks of the continental crust. The time terms for individual shots and stations in this region are of the order of 0.3 to 0.6 s, which are consistent with a varying thickness of between 2.5 and 5.0 km of lavas of average velocity 4.9 km s^{-1} overlying a basement of velocity 6.1 km s^{-1} . Alternatively a thinner lava sequence may be separated from the basement by pre-Tertiary sediments forming a low velocity layer.

Arrivals of P_g waves from shots to the south and east of the Faeroe Islands yield apparent velocities as follows: line B (shot times corrected for known sediment thicknesses): DU4– $5.28 \pm 0.02 \text{ km s}^{-1}$, F2– $6.00 \pm 0.08 \text{ km s}^{-1}$ from 0 to 50 km and $5.31 \pm 0.04 \text{ km s}^{-1}$ from 50 to 110 km; line C: DU4– $5.52 \pm 0.08 \text{ km s}^{-1}$, F2– $4.97 \pm 0.04 \text{ km s}^{-1}$, F6– $5.38 \pm 0.06 \text{ km s}^{-1}$. A time-term analysis on all the data here yields a refractor velocity of $5.53 \pm 0.08 \text{ km s}^{-1}$. The arrivals from line B recorded at station F2 (Fig. 3) are particularly significant, indicating that the 6 km s^{-1} basement beneath the northern region gives way to a lower velocity basement which occurs to the south-east of a line very approximately passing beneath shot 3 of line B in a NE-SW direction. The 5.3 to 5.5 km s^{-1} basement refractor to the south-east of this line could be interpreted as a folded series of shales, greywackes, slates and so on striking in a north-east to south-west direction. No higher velocity first arrivals attributable to P_g have been observed, implying that the 5.4 km s^{-1} layer must extend to substantial depth.

Shots on lines B and C on the Faeroes shelf beyond a distance of about 110 km give rise to the Moho head wave P_n observed at stations DU4 and F2 on the Faeroe Islands (Fig. 3). This data by itself is not adequate to determine satisfactorily the crustal structure of the Faeroe Block, but fortunately good P_n arrivals from shots on the northern and eastern parts of the Faeroe shelf were observed at the Shetland array station. We have therefore carried out a time-term analysis of P_n arrivals at stations DU4 (=B1), F2 (=C54B) and UKAEA for the following shots: line A 1–6, line B 1–16, line C 54A, 54C and 55–59. The resulting estimate of P_n is $8.24 \pm 0.35 \text{ km s}^{-1}$ and, excepting an anomalous low value of 2.83 s at shot A1, all the time-terms on the Faeroe Block lie between 3.15 and 4.00 s. The mean time-term is 3.60 s which yields a mean crustal thickness of 33 km for an average crustal velocity of 6.1 km s^{-1} , or 40 km for 6.6 km s^{-1} . The mean time term for the stations and shots in the immediate vicinity of the Faeroe Islands (that is, stations DU4 and F2, shots A1–3, C54A, C54C, C55 and C56)

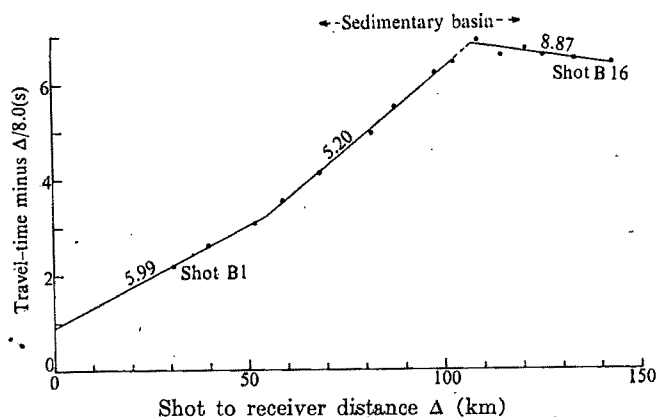


FIG. 3 Reduced time-distance graph for shots B1 to B16 at station F2 on the Faeroe Islands.

is 3.45 s, yielding crustal thickness estimates of 31 km for 6.1 km s^{-1} and 38 km for an average crustal velocity of 6.6 km s^{-1} . If, on the other hand, the true P_n velocity is 8.0 km s^{-1} , then the average time terms are slightly reduced and the resulting crustal thickness estimates are about 5–10% lower than those given above.

Although the velocity of P_n is not particularly well determined, the time terms are determined with some confidence. Depending on assumptions of true P_n value and mean crustal velocity, the crustal thickness beneath the Faeroe Islands is estimated to lie between about 27 and 38 km. There is no evidence for a 6.4 to 6.8 km s^{-1} layer at fairly shallow depth, such as gives rise to clear first arrivals on Iceland and the northern part of the Iceland-Faeroe Ridge, but this does not exclude the possibility that such higher velocities occur in the lower part of the crust.

Our results show that the crust beneath the Faeroe Block differs in four important respects from the anomalous oceanic crust beneath Iceland¹⁰ and the Iceland-Faeroe Ridge⁴: (1) P_n velocities of 5.9 to 6.2 km s^{-1} and less are characteristic of the Faeroes but velocities within this range have not been observed beneath Iceland; (2) a 6.4 to 6.8

km s^{-1} layer (oceanic crustal layer 3?) is present beneath Iceland and the Ridge but has not been observed at a shallow depth beneath the Faeroes; (3) a high sub-Moho P_n velocity of 8.2 km s^{-1} is observed for the Faeroes, in contrast to the anomalously low value of 7.2 km s^{-1} beneath Iceland; (4) the Faeroes' crustal thickness of about 30 km is substantially greater than that beneath Iceland. Our results thus seem to confirm that continental crust underlies the Faeroe Islands and the adjacent shelf regions. This suggests that the Rockall-Faeroe Plateau as a whole may form a single microcontinental fragment which originated as a result of the early stages in the evolution of the north-eastern North Atlantic, with subsequent strong subsidence affecting parts of the region between Rockall Plateau and the Faeroes.

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Direct physical evidence for secondary structure in an isolated fragment of R17 bacteriophage mRNA

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The region of R17 RNA protected from RNase digestion by coat protein assumes a stable, predictable secondary structure. It seems that this structure is recognised by the protein when functioning as a translational repressor.

NUCLEOTIDE sequence analysis of naturally occurring RNA molecules generally reveals sequences which may be arranged into plausible secondary structures. In the case of the RNA bacteriophages, specific structures have been suggested for many segments of the single-stranded genome, including the three ribosome binding sites¹, extracistronic regions at the 5' and 3' termini²⁻⁶, and the phage coat pro-

tein cistron⁷⁻⁹. Recently, Bernardi and Spahr¹⁰ characterised a 59-nucleotide fragment from R17 RNA which is specifically protected from nuclease digestion in the presence of coat protein. Thermodynamic rules for the evaluation of RNA secondary structures¹¹ predict a structure for the fragment consisting of two very stable hairpin helices (see Fig. 1). We have investigated the secondary structure of the isolated fragment by physical techniques and find close agreement between the observed properties of the RNA and those expected based on the proposed secondary structure. Magnetic resonance measurements reported in an accompanying paper give further strong support for secondary structure¹².

Our measurements differ from a conventional melting curve in that we resolve the time dependence of the melting process after a temperature perturbation. The data are obtained as oscilloscope traces^{11,13} which display both the time dependence and the total absorbance change. The latter can be plotted as a function of average cell temperature (average of initial and final values) for a constant temperature-jump size. The result (Fig. 2) is a derivative of the standard melting curve. The presence of two maxima in the derivative curves indicates that melting occurs in (at least) two steps, as would be expected for the secondary structure in Fig. 1.

TABLE 1 Stability of RNA secondary structure

	T_m in 0.05 M Na ⁺	T_m in 8 mM Mg ²⁺	T_m adjusted 1 M Na ⁺	ΔG (25° C)	ΔG (61° C)	ΔG (83° C)
Hairpin <i>b</i>	48° C	64° C	61° C	-5.0 kcalorie-mol ⁻¹	0 kcalorie-mol ⁻¹	+3.2 kcalorie-mol
Hairpin <i>a</i>	70° C	88° C	83° C	-11.4 kcalorie-mol ⁻¹	-4.5 kcalorie-mol ⁻¹	0 kcalorie-mol

The experimental T_m values were adjusted to 1 M Na⁺ by assuming an increase in T_m of 13° C for an increase by a factor of 20 in Na⁺ concentration. This increment is the average of the estimated minimum (8° C, from ref. 14) and maximum (17° C, in 8 mM Mg²⁺, Fig. 2c) variation. ΔG values refer to 1 M Na⁺.

The time resolution of the absorbance change indicates that each maximum in the differential curve corresponds to melting of a single helix, because the absorbance change separates into just two relaxation components. One of these is very fast, less than a few microseconds, and is nearly constant in amplitude between 40° C and 80° C. This is the usual behaviour for unstacking of bases in single-strand regions, and for temperature variation of the double helix absorbance^{11,13}. The other relaxation component is slower, can be described by a single exponential decay, and its amplitude peaks sharply at the maximum of the differential curve; relaxation times are shown in Fig. 3. These properties are characteristic of the melting of model double

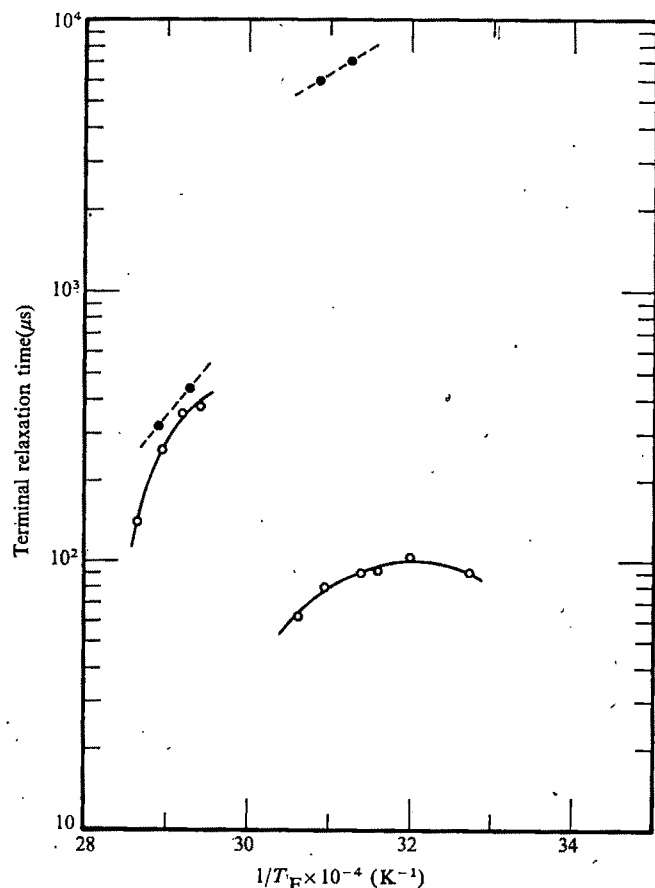


Fig. 3 Measured terminal relaxation time (τ), plotted logarithmically versus reciprocal of final temperature. Open circles were obtained in buffer I described in Fig. 2. All relaxation could be fit to a single exponential. Closed circles were obtained in the same buffer with added coat protein as described in Fig. 2b. Only four points are shown since the relaxations were difficult to analyse quantitatively over the entire temperature range. The data could be fitted to a single terminal exponential, but an additional exponential of approximately 100 μ s was also present in the lower temperature transition.

helices^{11,13-16}. If two double helices were melting at one of the differential maxima, they would have to have identical kinetic properties and identical T_m values at two salt concentrations in order to give a single relaxation component. Such a coincidence is sufficiently unlikely that it can be discounted.

The T_m and enthalpy of melting for each of the two helices can be extracted from the differential melting curve by methods described previously^{11,13}. The results are shown in Tables 1 and 2, where the heat and thermal stabilities are compared with values expected for helices *a* and *b*, based on our published thermodynamic parameters for model helices¹¹. Agreement of observation and prediction is good if the lower temperature transition is assigned to hairpin *b*. This assignment is strongly supported by the NMR results in an accompanying paper¹².

Table 1 also includes an estimate of the free energy of forming each helix at several temperatures, calculated from the measured heat of melting and the T_m corrected to 1 M Na⁺. At 25° C, secondary structure formation is strongly favoured; we estimate that the fraction of molecules in which helix *b* is unbonded at 25° C (1 M Na⁺) is about 2×10^{-4} and even less for helix *a*. Therefore we conclude that the RNA fragment forms a stable secondary structure, and the pattern of thermal unfolding is that expected for the two hairpin helices predicted from the sequence. We find no evidence for any additional stable structure with appreciable hypochromism.

Combination of the differential melting curve with the relaxation times allows calculation of forward and reverse rate constants for each helix^{11,13,15} (Fig. 4). The observed rate of helix formation is somewhat slower than is found for some model hairpin helices^{11,14}, but not unusual when compared with values found for tRNA^{17,17a}.

Binding of coat protein

When intact R17 RNA is incubated with coat protein, the formation of a complex can be detected by sedimentation in sucrose gradients²¹. Estimates of the number of protein molecules bound per RNA range from one to six^{18,21}. Since coat protein is a known inhibitor of replicase expression, it was suggested that the bound protein interferes with translation by directly preventing the attachment of ribosomes to the beginning of the replicase gene¹⁰. We have shown that the fragment protected by coat protein (Fig. 1) assumes a stable secondary structure, and we can now ask

TABLE 2 Comparison between predicted and experimental thermodynamic parameters

	Experiment		Prediction	
	T_m	ΔH	T_m	ΔH
Hairpin <i>b</i>	61° C	-47 kcalorie	59° C	-56 kcalorie
Hairpin <i>a</i>	83° C	-70 kcalorie	80° C	-80 kcalorie

Parameters refer to 1 M Na⁺.

whether the interaction with coat protein alters its physical properties.

First, we showed that the coat protein rebinds to the isolated fragment and determined the stoichiometry of the interaction. The apparent molecular weight of the complex as a function of added coat protein was measured by sedimentation equilibrium (Fig. 5). The weight average molecular weight of the RNA reaches a plateau value after the addition of 1 mol coat protein per mol RNA. Agreement between the observed molecular weight and the actual value (both the nucleotide and amino acid sequences are known) requires that the unknown effective partial specific volume (\bar{v}) of the complex be 0.74. The end point of the binding curve clearly indicates that on the average a 1:1 complex is formed, as has been reported previously for the whole RNA containing this sequence¹⁸.

Does the presence of bound protein affect the melting of the RNA secondary structure? Temperature-jump melting curves were obtained for the RNA-protein complex (Fig. 2b). The kinetics for the terminator helix *a* are unchanged, but strikingly, the rate of melting the initiator hairpin *b* is 100 times slower in the complex (Fig. 3). Coat protein therefore must interact with hairpin *b* in a manner which slows its melting. Although the terminator hairpin kinetics are unchanged, we cannot positively exclude the possibility of its interaction with coat protein since even the bound

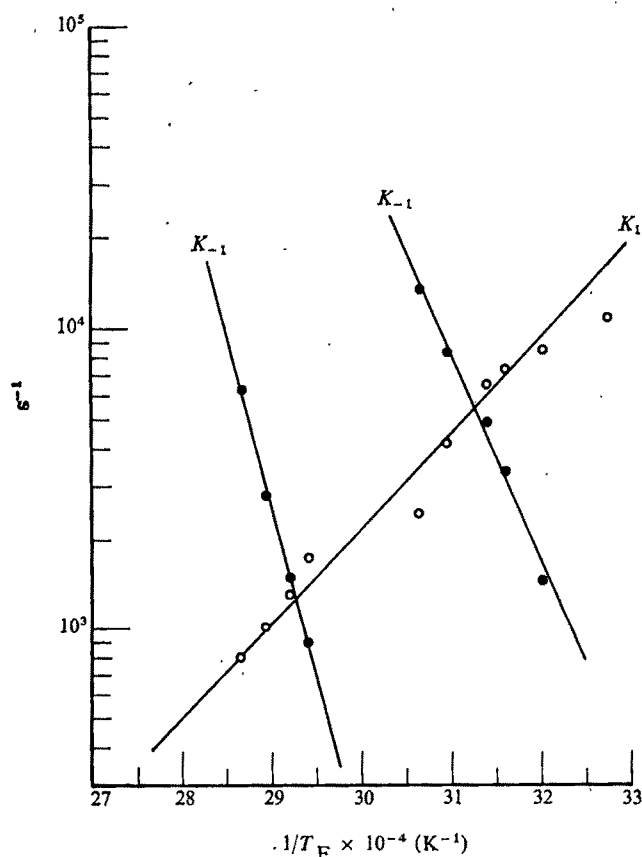


Fig. 4 Arrhenius plot of the temperature dependence of the RNA formation (k_1) and dissociation (k_{-1}) rate constants in buffer I. Resulting dissociation activation energies are +32 kcalorie for the lower transition and +54 kcalorie for the upper; the formation activation energy is -14 kcalorie in both cases. Rate constants were calculated as follows: the T_m and ΔH corresponding to each transition were determined¹⁸ from the peak position and width of the corrected differential melting curve (see Fig. 2), allowing calculation of the equilibrium constant K at any temperature. Knowledge of the relaxation times (Fig. 3) is then sufficient to calculate the rate constant by the equations $K = k_1/k_{-1}$ and $1/\tau = k_1 + k_{-1}$.

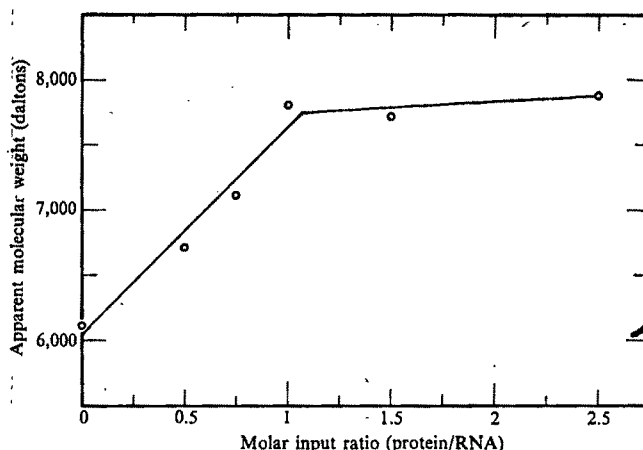


Fig. 5 Apparent molecular weight of the RNA-protein complex in buffer I as a function of added coat protein. Molecular weights were measured at 23° C and 266 nm in a model E analytical ultracentrifuge equipped with ultraviolet scanning optics. These were calculated in the standard manner, where M (apparent) = $(2RT/\omega^2)/(\partial \ln c/\partial x^2) = (1 - \bar{v}\rho)M$ (true), where \bar{v} is an effective partial specific volume that also accounts for polyelectrolyte effects. Each point represents a run where previously unused RNA was mixed with coat protein at the indicated relative molar concentrations. The concentration of RNA was always 0.66 μ M at 260 nm. Plots of $\log c$ against x^2 were linear within experimental error. Under the conditions used, the absorbance is predominantly that of the RNA, so M (apparent) is only slightly affected by the presence of free protein.

protein might become denatured at this higher melting temperature. The melting temperature (within experimental error) of neither hairpin is altered upon complex formation (Fig. 2b). This observation by itself could mean that the protein binds equally well to the hairpin helix *b* and the single strand, or that the free energy of binding is nearly zero at the T_m , or that an irreversible process, presumably involving protein denaturation, occurs at the T_m . We favour the last explanation because of the thermal instability of the isolated R17 coat protein.

What aspect of RNA structure is responsible for specific recognition of the fragment by R17 coat protein? We have found that (1) on the average one coat protein is bound per RNA molecule (Fig. 5); (2) the RNA is recognised while it assumes a stable secondary structure (Fig. 1); (3) the rate of melting of the helix which includes the initiator triplet is slowed in the RNA-protein complex (Fig. 3); (4) T_1 digestion of the R17 RNA-coat protein complex sometimes yields only the initiator hairpin in the protected fragment but never only the terminator hairpin (Fig. 1); (5) coat protein will not rebound the T_1 RNase-digested fragment¹⁹; (6) coat protein will specifically bind the isolated replicase initiator region¹⁹, which includes only hairpin *b* of the fragment in Fig. 1.

We conclude that coat protein recognition most likely requires some aspect of the RNA secondary structure, rather than simply a single-strand sequence. The structure which is recognised includes the ribosome binding site at the beginning of the replicase gene. Assuming that a similar structure is formed in the intact R17 RNA molecule, the direct interaction of coat protein with this region would provide a molecular explanation for the translational repression.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Are binary systems involved in supernova explosions always disrupted?

No known pulsar is a member of binary system. Does this mean, as has been suggested¹, that pulsars are the result of supernova explosions (hypothesis 1), and explosions which occurred in binary systems have in most cases disrupted the systems (hypothesis 2)? The discovery of two X-ray 'pulsars' (Cen X-3 and Her X-1) and other compact X-ray sources in binary systems seems to be an argument against hypothesis 2 if we believe that hypothesis 1 is also valid for compact X-ray sources. Here I show that hypothesis 2 is indeed untenable from the point of view of present theories of close binary evolution.

A binary system with period of several years may be close enough for the original primary to lose a considerable amount of its mass through the mass transfer in the late stage of its evolution². Van den Heuvel³ shows that 80% of the known OB spectroscopic binaries will undergo case B mass transfer (during hydrogen shell burning). Therefore it is reasonable to believe that supernova explosions in close binary systems are mostly preceded by mass transfer.

It is interesting that all known massive X-ray binaries have a common property that they consist of an OB supergiant with mass of about $30 M_{\odot}$ and a compact object with mass in the range between ~ 0.5 and $\sim 5 M_{\odot}$ (ref. 4). In five of the six known X-ray binaries the compact object has $M < 3 M_{\odot}$; only in Cyg X-1 might it be more massive. The large mass ratio of these massive X-ray binaries supports the hypothesis that the explosion is more likely to have occurred after the mass transfer. In that case the exploded star is more likely to be the less massive component⁵.

I assume that the mass of the original secondary (presently the primary) is constant during the explosion. The explosion is supposed to be spherically symmetric and to take place instantaneously. I also assume that initially the orbit is circular. Adopting these assumptions and taking the effects of the impact of the supernova shell onto the unexploded component into account in the way formulated by Colgate⁶, one can show that the condition for a binary

system to remain bound after a supernova explosion is⁷

$$\alpha + 2\beta - \gamma - 1 > 0, \quad (1)$$

where $\alpha = M_2/M_1$, $\beta = M_1'/M_1$ and γ is the ratio (gain in kinetic energy of the unexploded star due to the impact)/(initial orbital energy). M_2 is the mass of the unexploded component; M_1 and M_1' are the mass of the exploded star before and after the explosion respectively.

I assume a typical value for the velocity of matter ejected by a supernova explosion is 10^9 cm s^{-1} (ref. 8). For a given combination of values of M_2 , M_1' and the initial orbital period P , one can determine from the inequality (1) the critical value of α (α_{cr}) below or equal to which the condition (1) is unsatisfied (that is, the binary will be disrupted). I take $M_2 = 30 M_{\odot}$ and $M_1' = 1$ and $5 M_{\odot}$ re-

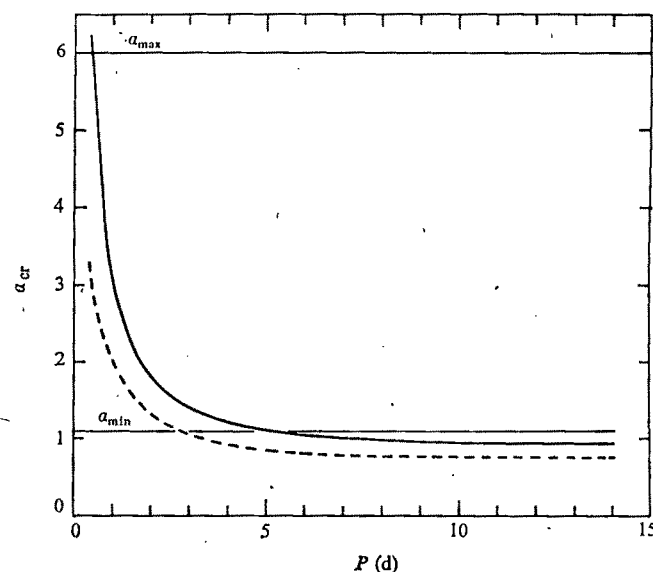


FIG. 1 The values of α_{cr} as a function of P for systems with final masses $30 + 1 M_{\odot}$ (—) and $30 + 5 M_{\odot}$ (---). For a given value of P the binary system will be disrupted if $\alpha < \alpha_{cr}$. The horizontal lines labelled α_{min} and α_{max} indicate respectively the lower and upper limit of the possible values of α (see text).

spectively. These values seem to be representatives for all massive X-ray binaries. The values of α_* as a function of P are given in Fig. 1.

It is likely that in massive X-ray binaries only a small fraction f of the transferred mass will escape from the system⁹. I assume that $f \lesssim 30\%$ which is consistent with the conclusions obtained from observations of evolved close binaries^{9,10}. If the explosion takes place after case B or case C mass transfer and if $f \lesssim 30\%$, the possible values of α are between ~ 1.1 and ~ 6 for $M_2 = 30 M_\odot$ (my unpublished work). From Fig. 1 one can see that all binaries with periods larger than about 5 d will remain bound after the explosion. Binaries with such periods form about 50% of the O-B5 spectroscopic binaries in Batten's Catalogue¹¹. Since, if the remnant mass is larger than $1 M_\odot$, also some binaries with a period of less than 5 d remain bound after the explosion, the probability of maintaining close binary systems (at least for close binaries with an O-B5 component) through supernova explosions should be considerably larger than 50%. Therefore the disruption of such binary systems involved in supernova explosions is more likely to be an exception rather than a rule.

Even if f is 40%, systems with $P \gtrsim 5$ d in which the remnant mass is $\gtrsim 2 M_\odot$ will remain bound. My results do not depend critically on the exact choice of a limit for f . So it seems necessary to seek another explanation for the non-existence of radio pulsars in binary systems. The absorption and smearing out of the radio pulses by the gas flows in the system might be such an alternative explanation.

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Internal rotation of the Sun and the solar neutrino flux

THE low upper limit of 1 SNU (10^{-26} captures per ^{37}Cl atom s^{-1}) on the observed neutrino flux from the Sun obtained by Davis¹ has proved an embarrassment to stellar physicists, and in spite of considerable intellectual gymnastics the standard solar models predict at least 6 SNU (ref. 2). The essential difficulty has been to produce a model with a low enough central temperature that can still produce the observed luminosity of the Sun with an age of 4.7×10^9 yr.

Arguments for a rapid central rotation, causing a low neutrino flux, are elaborated below.

The equations determining the structure of a non-uniformly rotating star can be averaged over spheres and, provided the rotation is not too large, approximate to^{3,4}

$$\frac{1}{\rho} \frac{dP}{dr} = -\frac{GM_r}{r^2} \rho(1 - \alpha), \quad \alpha = \frac{2}{3} \left(\frac{\Omega^2 r^3}{GM_r} \right)$$

$$\frac{dM_r}{dr} = 4\pi r^2 \rho$$

where the symbols have their usual meaning.

The remaining stellar structure equations can be similarly averaged but are the same as the non-rotating equations to order α^2 . If α , the ratio of centrifugal force to gravity were constant throughout the star we could write $G^* = G(1 - \alpha)$ and the rotating structure equations are identical to the equations governing spherical star, in particular the relative variation of the physical variables is unchanged (that is T/T_c in terms of r/R for example), although their absolute value would be changed. Simple homology arguments then give

$$L \propto \frac{(\mu G)^8}{X^{1.2}}, \quad T_c \propto \frac{(\mu G)^2}{X^{0.4}}, \quad \mu = \frac{4}{(3 + 5X)}$$

where X is the fraction by mass of hydrogen; so that for $L = \text{constant} = L_\odot$, T_c changes negligibly and Σ_2 is only very slightly increased. On the other hand, if the angular velocity is constant α increases outwards and by scaling the models of Faulkner, Roxburgh and Strittmatter⁴ we see that for fixed L , T_c increases and Σ_2 increases more substantially.

These results, which are borne out by accurate calculations (to be published), suggest trying models where α decreases outwards; in such models the relative variation of the variables is changed—the temperature profile for example, is flattened, (Fig. 1). For such models we may expect a decrease in central temperature for a fixed luminosity and hence a decreased neutrino flux.

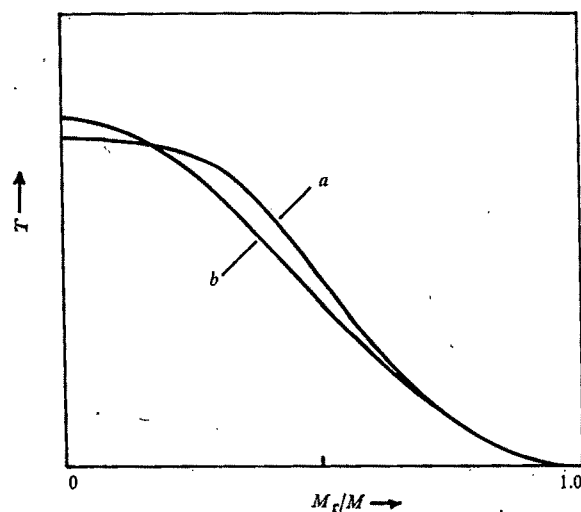


Fig. 1 The effect of non-uniform rotation on the temperature distribution inside stars. a , $\Omega^2 r^3/GM_r$ decreases outwards; b , $\Omega = 0$.

The Sun, of course, has evolved for some 4.7×10^9 yr and in inhomogeneous evolved models there are two factors lowering the neutrino flux compared with the standard models: first, the change in the temperature profile demonstrated previously; second, the increased homogeneity as compared to models with $\alpha = 0$ due to the increased region of nuclear burning caused by the changed temperature gradient. Detailed calculations show that these effects can reduce the

neutrino flux for the present Sun to less than 1 SNU (Table 1).

TABLE 1 Inhomogeneous solar model ($t = 4.7 \times 10^9$ yr; $L = L_Z/X = 0.019$)

	X_c	$\log T_c$	Σ_ϕ
$\alpha = 0$	0.35	7.17	6.5
$\alpha = \alpha^*$ (see Fig. 3)	0.50	7.11	0.93

In passing I note that it is no surprise that the calculation by Ulrich⁵ gave no substantial decrease in the neutrino flux. In his model Ω was constant and therefore α increased outwards in a core. It then dropped discontinuously to zero. We would not expect the right change in temperature profile from such a model.

The angular momentum per unit mass of interstellar gas clouds is so large that if a star forms it is highly probably that it will be rapidly rotating. Subsequent contraction exacerbates the problem; the central regions would spinup leading either to dynamic instabilities and possibly fission⁶

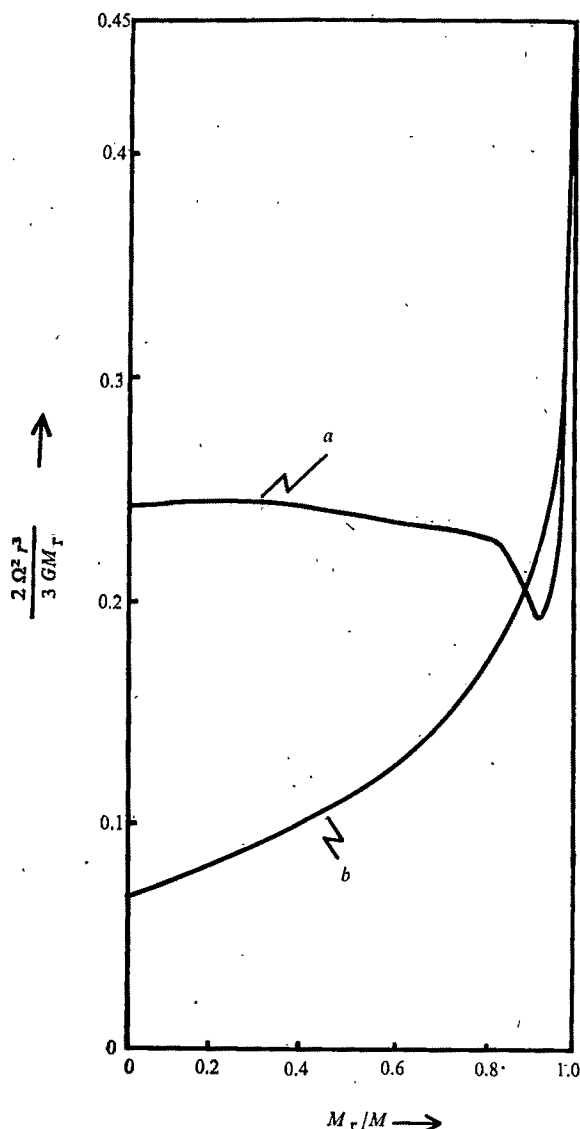


Fig. 2 Ratio of centrifugal force to gravity in a solar model before and after pre-main sequence contraction. *a*, End of contraction; *b*, Beginning of contraction.

or just a dynamically stable rapidly spinning core. Detailed calculations of contracting solar models including the effect of rotation have been made by Williams⁷ and in Fig. 2 I show the ratio of centrifugal force to gravity, α , after contraction. This angular velocity distribution is not stable to thermal perturbations, that is, those where radiative gains compensate for the stable stratification so that the fluid responds like an incompressible liquid^{8,9}. The instabilities on a scale l have growth rate given by

$$\tau = \tau_{th}(l/R)^2 1/\alpha, \quad \tau_{th} = 3GM^2/(4RL)$$

where τ_{th} is the thermal response time of the star $\sim 2 \times 10^7$ y. Since the Reynolds number is so large we must expect these instabilities to be turbulent and we can then estimate the mixing time by assuming a random walk process, requiring $(R/l)^2$ steps of length l . The mixing time is then the same for all models and is τ_{th}/α or about 10^8 yr.

During this time the chemical composition has changed due to the central burning of nuclear fuel, so there is now a chemical gradient which can stabilise an angular velocity gradient.

The angular velocity therefore adjusts initially to a marginally stable state in one or two mixing times and then continually readjusts as the Sun is slowed down by angular momentum loss in the solar wind. In the first stage there has to be substantial mass loss of about $0.3 M_\odot$. The results of detailed calculations are given in Fig. 3; the centre of the Sun is left spinning rapidly while the bulk is slowed down, α decreases outwards and this gives the low neutrino flux.

The external quadrupole moment has a value of about $J_2 = 6 \times 10^{-5}$, comparable with Dicke's measured value. The central region spins at a phenomenal rate with a period of about 50 min—it would be of considerable interest to look

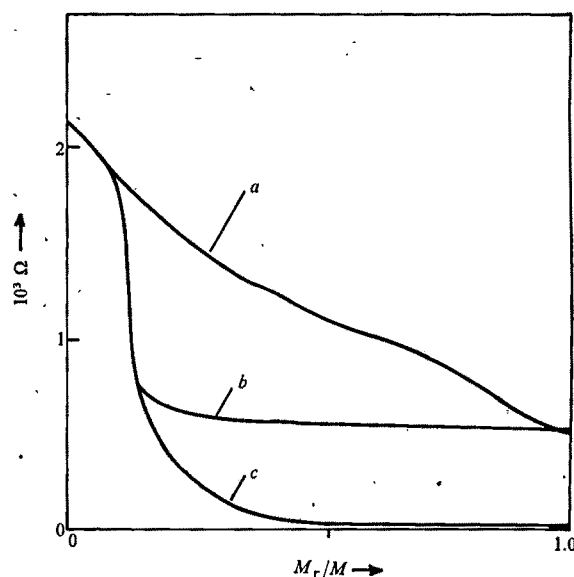


Fig. 3 Angular velocity inside the Sun: *a*, after contraction; *b*, after re-adjustment; *c*, after solar wind braking. *c* is the distribution Ω^* used in the text.

for phenomena on the Sun with a period of 30 min to 1 h that might reflect this rapid motion; if it exists it might lend support to the "rapid core" explanation of the low neutrino flux.

Care should, however, be exercised before concluding that these models support the scalar tensor theories of gravity. In those theories the constant of gravity decreases in time which accelerates the nuclear burning and so increases the central temperature increasing the neutrino flux.

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Light curves of very faint meteors

THE variation of brightness as a function of height when a meteoroid burns up (ablates) in the Earth's atmosphere is governed by the physical properties of the meteoroid. The basic theory of the ablation of a solid meteoroid due to Öpik¹, and the theoretical light curves are in fairly good agreement with the observed light-curves of very bright meteors². In contrast to this the observations of Jacchia³ and Hawkins and Southworth⁴ of faint photographic meteors show that the train lengths of these meteors are much shorter than predicted by the classical theory, and it is principally due to evidence of this sort that the "dustball" theory of Whipple⁵ which supposes the original meteoroid to consist of an aggregate of many small solid particles, has become very popular. Nevertheless Jones and Kaiser⁶ found that by extending the classical ablation theory to include the effects of the meteoroid's thermal capacity, conduction and radiation they could explain many features of the experimental data in terms of compact meteoroids which fragment as a result of thermal shock. According to Jones and Kaiser⁶ it is possible to choose between the two theories on the basis of observations of very faint meteors (magnitude $M > +3$) since these meteoroids should not fragment due to thermal shock if they are compact; on the other hand if they are fragile conglomerates they will probably continue to show the effects of severe disintegration.

To this end we have developed an observing system⁷ using sensitive television equipment which has a limiting sensitivity of $+8.5$ mag for stars when operating with an $f/0.95$, 50 mm lens.

On the night of September 31/October 1, 1973 in London, Ontario, 179 meteors were observed with the system between 11 p.m. and 6 a.m. with the camera pointing north at an elevation of 50° .

Analysis of the video tapes enabled the train lengths perpendicular to the line of sight to be determined assuming the meteors to occur at an altitude of 95 km. The apparent train length l_a is related to the height interval between the starting and finishing height dh by the relation

$$dh = r l_a$$

where r is a random variable depending on the geometry of the camera and the meteor trains. Since at that time of year the activity of meteor showers is expected to be low, we tried two assumptions in order to estimate the mean value of r ; a random distribution of radiant and also a point radiant such that all the meteors were supposed to come from the apex of the Earth's way. Fortunately each of these assumptions yielded a mean value of r very close to unity.

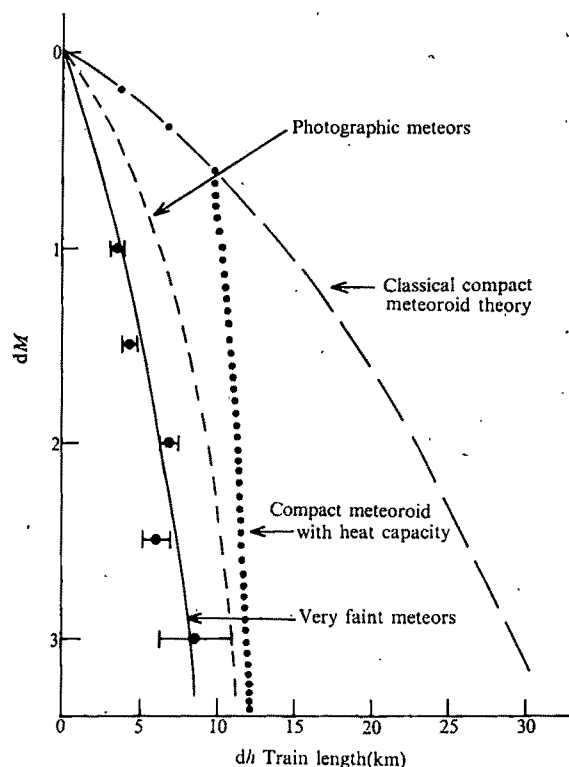


Fig. 1. The difference dM between the magnitude of the meteors and the limiting magnitude of the observing system plotted against dh , the height interval between the starting and finishing height of the meteors.

In this way we obtained the results in Fig. 1 which shows dh as a function of dM , the difference between the magnitude of the meteor and the limiting magnitude of the system. For the sake of comparison we have also shown the theoretical predictions for the compact meteoroid model and the curve of dh against dM computed using the photographic data of Hawkins and Southworth.

It is clear that not only are the train lengths of the television meteors considerably shorter than predicted by the compact meteoroid model but they are also significantly shorter than those of the photographic meteors. We interpret this as indicating that fragmentation is even more severe for the very faint meteors than for the meteors of Hawkins and Southworth observed using Super-Schmidt cameras. On the basis of these results we conclude, albeit reluctantly, that the compact meteoroid model is no longer tenable.

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Strong long-period tropospheric and stratospheric rhythm in the Southern Hemisphere

THE recent EOLE satellite-balloon atmospheric sensing experiment conducted in the Southern Hemisphere between August 1971 and July 1972, provided large quantities of upper tropospheric data in a hitherto near-dataless region. Besides measurements of ambient temperature and pressure reported directly from the balloon to the satellite, the experiment allowed the calculation of series of highly accurate Lagrangian velocities by the positioning of the balloon between successive satellite orbits. The experiment and data are described in detail by Morel and Bandede¹.

To obtain a Eulerian data set, the Lagrangian data required transformation. As no general analytical transformation exists², the Eulerian information was obtained by computing daily averages of all the measured Lagrangian quantities in each area of 10° latitude by 20° longitude. The resulting average was assumed to be the representative Eulerian daily average of that quantity. The rationale and justification of this method is given elsewhere (ref. 3 and P.J.W., D. G. Curtin and Y. Mintz, to be published).

Tropospheric rhythms: The many attempts to find a distinct atmospheric periodicity on a global or hemispheric scale have met with little success. Most notable are the efforts of Willett⁴ and Namias⁵ in the Northern Hemisphere and recently by Taljaard⁶ in the Southern Hemisphere. All studies defined some index which was more or less sensitive to the change of state of the atmosphere. Although large variations in the various indices did occur, no dominant rhythm was identified⁶.

Another common feature of these studies was the use of land based Eulerian sensors which, on account of their geographical grouping, may not have been suitable for the calculation of a representative hemispheric index. To overcome this problem, the Eulerian data inferred from the dense EOLE data set was used to define a zonal index sensitive to the transition of the atmosphere from perturbed to a zonal state. Such an index (R) is defined as the ratio of the kinetic energy of the mean flow (K_z) and the zonal average of the perturbation or eddy kinetic energy (K_E), that is,

$$R = K_z / K_E = \frac{1}{2 \cos \phi} \int_0^{2\pi} (\bar{V} - [\bar{V}])^2 d\lambda / ([\bar{V}]^2 / 2)$$

where

$$[\bar{V}] = \frac{1}{\cos \phi} \int_0^{2\pi} \bar{V} d\lambda.$$

Here λ represents longitude, ϕ latitude and \bar{V} the distribution of the daily mean Eulerian velocity inferred from the EOLE data.

Time sections of R are shown in Fig. 1 for three 10° latitude bands between 30° and 60°S. Large amplitude variations may be seen with an apparent period of some 20 d, especially in the higher latitudes. These represent regular changes from highly perturbed states (large R) to highly zonal states (small R). Superimposed upon this trend are variations of higher frequency and smaller amplitude indicating similar but short lived variations.

The times series spectral analyses of $R(t)$, shown in Fig. 2, tend to support these observations. In the 50°–60°S latitude spectra for R , a strong isolated peak suggests a strong rhythm centered near 20 d in the high latitudes, a feature which is also reflected in the K_E spectra. As illustrated by the 40°–50° spectra of R , the peak is somewhat weaker and further diminishes into the subtropics. Spectra of the zonally averaged pressure (lower diagram) indicates a weaker periodicity near 20 d, whereas the zonally aver-

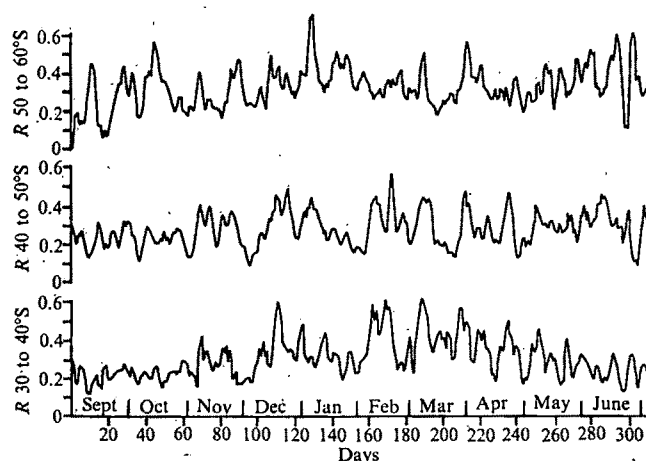


Fig. 1 Variation with time of the index R over the EOLE period for the three latitude bands indicated.

aged temperature field does not exhibit relatively as large a variation. This is common to the temperature field at all latitudes.

The cross spectra between the two component energies of $R(t)$ indicate an extreme peak near 20 d. An important feature is that, in the 50° to 60° band, K_E and K_z are almost exactly out of phase (-176°) and have a coherence of 0.825 in the 18 to 23 d band, surpassing the 99.9% confidence limit at this spectral band width. (Coherence between the various values of R for the different latitudes each

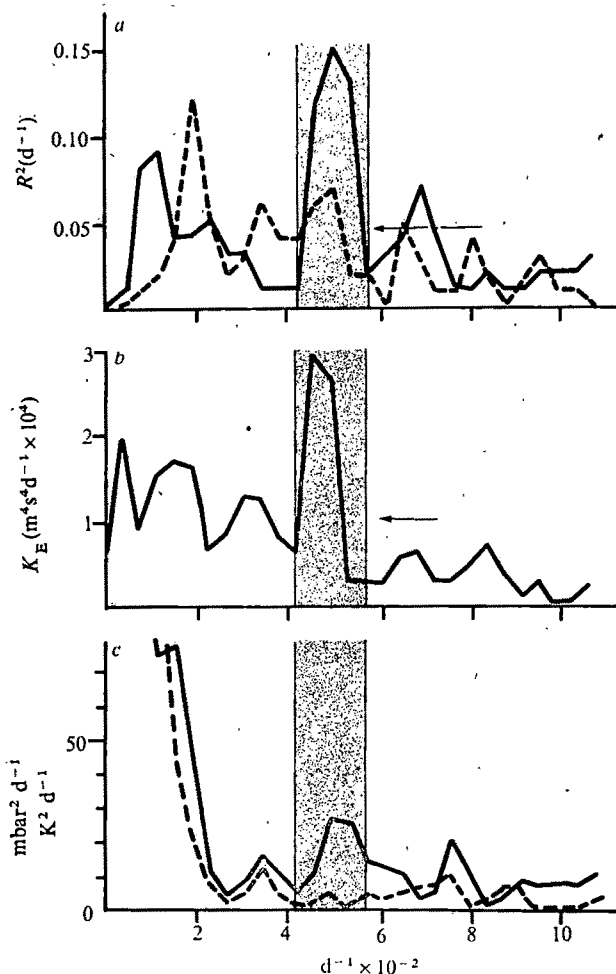


Fig. 2 Variance spectra. *a*: —, R for 50° to 60°S; ---, R for 40° to 50°S. *b*: K_E for 50° to 60°S. *c*: —, P ; ---, T . Both for 50° to 60°S. Arrows denote 90% confidence level, shaded region the 18 to 23 d band.

surpasses the 99% level in this frequency band.) Such phase and coherence strongly suggests that the quasi-20-d rhythm in R may be accounted for by the conversion of kinetic energy between that of the eddies and that of the mean flow, such that the cycle consists of waves growing at the expense of the mean flow and then the reverse conversion.

Spectral analysis in space and time indicates that much of the power of the 20-d rhythm is in the ultra-large scale waves. For example, in the 50° to 60° band, the kinetic energy in longitudinal wave numbers 1 and 2 is an order of magnitude greater in the 18 to 23 d period interval than for any other wavenumber. The momentum flux also shows a 20-d rhythm, which, together with the above, suggests that this variability in R is a manifestation of a slow period and very large scale barotropic energy conversion between K_n and K_z which is similar in character to the barotropic energy conversion described by Lorenz⁷. But this process is generally thought to be restricted to a much smaller scale ($\sim 1,000$ km) and higher frequency motions (~ 4 to 5 d) and is considered to be of secondary importance in the energetics of the general circulation.

In contrast, we suggest that the precominant process in upper-troposphere of high latitudes over the time scale of weeks is the barotropic exchange of energy between the ultra-long waves and the zonal flow.

(ii) Stratospheric rhythms: Initial attempts have been made to determine to what extent the stratosphere is effected

by the large scale, long period tropospheric oscillations suggested above. Due to the scarcity of conventional data and the consequent inability to construct a zonal index for the stratosphere, total ozone data from the few scattered Southern Hemisphere stations were analysed.

Figure 3 shows the time series spectral analyses of the total ozone for the three stations, Argentine Island (65°S), MacQuarie Island (55°S) and Brisbane (28°S) chosen for the initial study on the basis of data availability and latitudinal spread. The only data modification was the removal of the dominating annual trend before the spectral analysis.

The common feature of all three spectra is the existence of power, to varying degrees, in the 18 to 23 d period band. This is especially evident at Argentine Island and MacQuarie Island, and surprisingly for a station so far equatorward, at Brisbane. Cross spectra between these ozone stations show strong coherence during the EOLE period in the 18 to 23 d band. The coherence between R and the stratospheric ozone is slightly weaker but this may be because we are seeking cross-spectra between point observations (the ozone) and hemispheric quantities (R).

The results presented here are not conclusive, but there seems to be a strong suggestion of a preferred and dominating physical mechanism existing over the period of weeks in the high latitudes of the upper troposphere of the Southern Hemisphere. This large scale and long period oscillation seems to be tied to the barotropic interchange of energy between the perturbations and the mean flow and possesses vertical propagation properties such that it may provide an important energy source for the stratosphere over this time scale.

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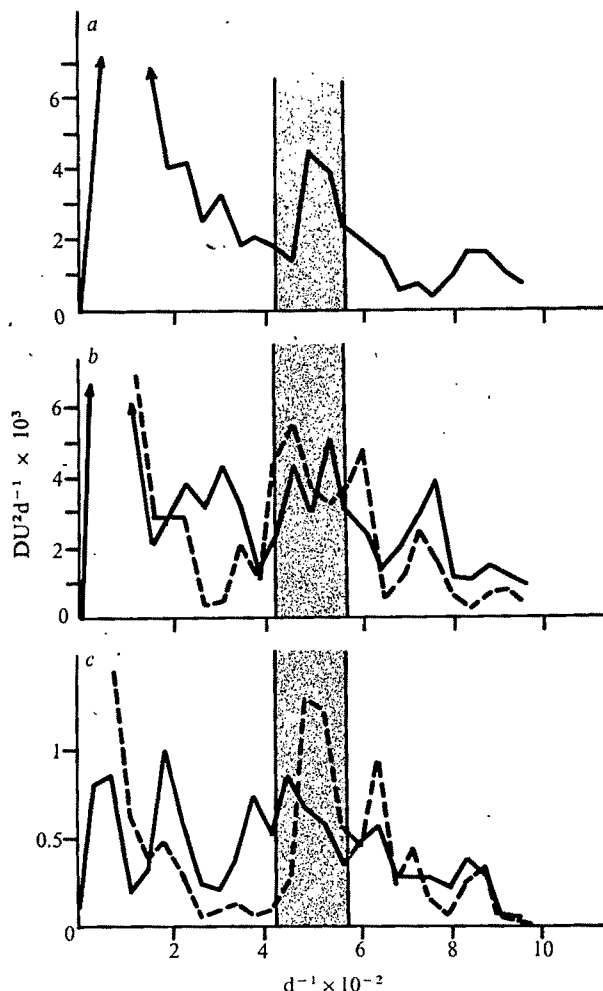


FIG. 3 Variance spectra of total ozone. a: Argentine Island (65°S), 1962-64. b: MacQuarie Island (55°S), 1971-72. c: Brisbane (28°S), 1971-72. Dashed lines denote data from EOLE period. 1 DU is equivalent to 10^{-3} cm NTP.

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Dispersion of ionospheric waves

THE phase speed dispersion of travelling ionospheric disturbances (TIDs) has been measured using data from a three-dimensional array of c.w. Doppler sounders¹. Horizontal and vertical trace speeds of phase surfaces were measured as a function of wave period by performing cross-spectral analysis of the array signals. Although the measured dispersion varied considerably depending on the coherence of the wave activity across the array, the average horizontal trace speed was approximately proportional to $T^{-1/2}$, where T is the period. From a very limited set of measurements, vertical trace speed was tentatively determined as proportional to T^{-1} . Additional measurements of highly coherent waves now indicate that the vertical dispersion is even stronger than T^{-1} at the short periods (12-25 min.).

Based on the reliably determined horizontal dispersion and a large diurnal variation of speed, I suggested¹ an

explanation of the observations in terms of hydromagnetic waves. This explanation did not account for the magnitude of the observed speeds but explained the horizontal dispersion and large diurnal variation of speed. Because of the difficulty of developing a satisfactory hydromagnetic wave explanation, I attempt here to show how the dispersion could be accounted for in terms of the widely accepted internal gravity wave explanation of TIDs (ref. 2).

The horizontal of phase speed gravity waves is a function not only of period but also of the tilt of the phase surfaces away from the vertical:

$$V_x = \frac{c}{\omega_0} [N^2 - \omega^2(1 + \tan^2 \phi(\omega))]^{1/2}$$

where N , ω_0 and ω are the Brunt, acoustic cutoff and wave frequencies, c is the speed of sound, and $\phi(\omega)$ is the tilt of the phase surfaces away from the vertical¹. As the phase surfaces tilt over towards the theoretical limiting angles of wavefront tilt, given by

$$\phi_L = \tan^{-1} \left[\frac{N^2}{\omega^2} - 1 \right]^{1/2}$$

the horizontal speed, V_x , approaches zero. When ϕ is zero (no tilt), or when ϕ is a fixed percentage of the limiting angle, V_x increases toward long periods. If, however, a wavefront tilt function, $\phi(\omega)$, is chosen in which phase surfaces tilt over closer to the limiting angles at long periods than at short periods, V_x can be made to decrease towards long periods. That is, the first equation above can be solved for $\phi(\omega)$ giving:

$$\phi(\omega) = \tan^{-1} \left[\frac{N^2}{\omega^2} - 1 - \frac{\omega_0^2 V_x^2}{c^2 \omega^2} \right]$$

For any specified horizontal phase speed dispersion, $\phi(\omega)$ will be less than ϕ_L and both will increase toward long periods with $\phi(\omega)$ approaching ϕ_L if V_x decreases toward long periods.

Figure 1 shows new observations of the mean horizontal and vertical phase speeds of ionospheric waves recorded between 1700 and 2200 LT on four successive days (November 22-25, 1972) in the New York City area. The horizontal speeds were determined from an array of four Doppler sounders operating at 4.5 MHz. The vertical speeds were determined from the phase lags of the downward-moving wavefronts between the reflection levels of 4.5 MHz and 2.5 MHz Doppler sounders. The absolute values of the vertical speeds are difficult to measure accurately because the difference in the reflection level of the two radio frequencies varies over the recording interval of 5 h. An average vertical separation of 20 km was however estimated using mean monthly electron density profiles for November 1972, at Wallops Island, Virginia, which is several hundred kilometers south of the array.

Both horizontal and vertical speeds decrease toward long periods. The observed phase speed dispersion can be best approximated by gravity wave theory if it is assumed that the waves propagate with the wavefront tilt given by the dotted line at the bottom of fig. 1. A Brunt period of 8 min was assumed which gives the limiting angle of tilt, ϕ_L , in fig. 1. The theoretical V_x and ϕ determine the theoretical V_z since $V_z = V_x / \tan \phi$. The theoretical speed decreases towards long periods because the theoretical value of ϕ approaches ϕ_L at long periods. The observed wavefront tilt is also shown. It does not entirely match the theoretical tilt function, but as required, is closer to the limiting angle at long periods. Either the observed V_x or V_z could be approximated more closely by a different theoretical tilt function but the best approximation to both is shown.

For the time of day of the observations, the radio signals from the Doppler sounders should have reflected near 200 km height, where the Brunt period is about 14 min for a

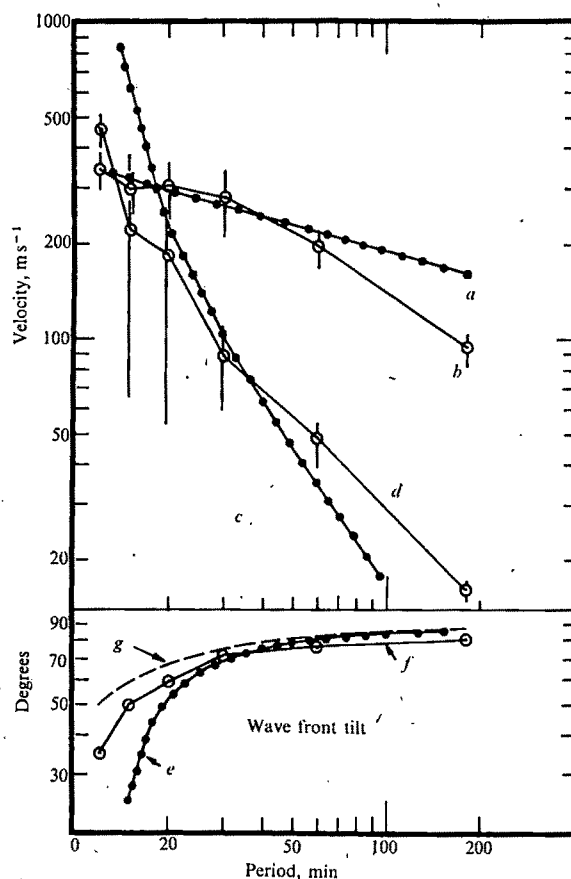


Fig. 1 Mean horizontal and vertical phase speeds and tilt angles of ionospheric waves recorded between 1700 and 2200 h LT on four successive days (November 22-25, 1972). Standard deviation bars are shown. Theoretical speeds and tilt function, consistent with gravity wave theory when the limiting tilt angle ϕ_L is assumed, are also shown. a, V_x theoretical; b, V_x observed; c, V_z theoretical; d, V_z observed; e, ϕ , theoretical; f, ϕ observed; g, limiting tilt angle.

1962 US Standard Atmosphere. The best fit of theoretical and observed curves was however, found using atmospheric parameters (N , ω_0 , c) that apply to about 125 km.

Theoretically, free interval gravity waves tend to propagate energy at increasing angles ($90^\circ - \phi_L$) about the horizontal, as period decreases³. If however, a broad spectrum of waves from a single source were observed at a receiving point, then the short period waves would necessarily have travelled at lower percentages of their limiting angles than did the long period waves, thus producing the apparent inverse dispersion. Figure 1 indicates that over a wide range of periods, the wavefront tilts need be only a few degrees less than the limiting angles to produce the observed dispersion.

A large diurnal variation of the phase speed of the F-region waves has been reported⁴. The speeds increase hundreds of per cent from day to night, maintaining the apparent inverse dispersion. Although it is difficult to explain why, I suggest that for some reason the waves simply propagate at angles further from the limiting angles at night, causing larger phase speeds at all periods.

It would be useful to use a very widely spaced array and attempt to measure group speeds of a sufficient number of waves to detect group speed dispersion and any diurnal variation of group speed. Such measurements are, however, probably much more difficult than accurate phase speed measurements. According to gravity wave theory, horizontal group speeds must be less than phase speeds. My suggestion¹ that the observed horizontal dispersion implies a group speed greater than the phase speed¹, would be invalid for the strongly anisotropic behaviour of gravity waves. As indicated here, the apparent inverse phase-speed dispersion could result

solely from the behaviour of the wavefront tilt and thus the slope of the phase-speed dispersion curve would give no information about group speeds.

Brownlie *et al.*⁵ have reported ionospheric wave measurements which suggest higher phase speeds at the longer periods, and they noted the discrepancy with the observations of Herron¹, such as shown in fig. 1. The speeds, however, vary considerably with time of day⁴, and if care is not taken to eliminate this effect, it could distort the dependence on period.

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Will another damaging earthquake occur in Koyna?

AN earthquake of magnitude 5.1 (Richter scale) occurred on October 17, 1973, near the Koyna Dam (17°23'N, 73°45'E) which experienced a damaging earthquake¹ on December 10, 1967. The former earthquake was preceded and followed by several foreshocks and aftershocks. Two of the foreshocks on the same day had magnitudes of the order of 4.0 and there was another significant earthquake of magnitude 4.6 in the same region on October 24.

It is now well known that filling of large artificial lakes behind high dams can trigger earthquakes²⁻⁴. The examples of Kariba, Kremasta and Koyna are particularly significant since damaging earthquakes of magnitude exceeding 6 have occurred in these regions.

Before the construction of the Koyna Dam in the western part of the Peninsular Shield of India no significant tremor had been recorded. Soon after the impounding of the Koyna reservoir in 1962, reports of earth tremors near the dam site began to accumulate. Thereafter thousands of tremors were located within 25 km of the dam by a network of four closely spaced seismological observatories⁵. Up to June 1969, 450 of these shocks had magnitude ≥ 3 . The earthquake of September 13, 1967 (magnitude 5.5) was the

first significant event and caused mild damage in Koyna Township. The earthquake of December 10, 1967 had a magnitude of 6.0, claimed about 200 lives and caused widespread damage. The earthquake of October 17, 1973 (magnitude >5) occurred in the Koyna region after a lapse of 5 yr, the last one having occurred on October 29, 1968. An analysis of the records of the Hyderabad seismograph station, situated about 490 km east of the Koyna Dam, shows that the frequency of earthquakes of magnitude ≥ 4.0 has decreased during the past few years following that of December 10, 1967. Table 1 shows that nine such earthquakes occurred in 1969, seven in 1970, four in 1971 and three in 1972. Between January and September 1973 only one earthquake of magnitude 4.1 occurred (on April 19). During October 1973 three earthquakes of magnitude >4 occurred in addition to the one of magnitude 5.1. The present activity does not fit the trend of gradually decaying seismicity in the Koyna region during the past few years.

From studies of reservoir levels and earthquake frequency at several places⁴ it has been inferred that, in addition to the tectonic setting and geological conditions, the factors affecting the tremor frequency near the reservoirs include (1) the rate of increase of water level; (2) the duration of loading; (3) the maximum level reached; and (4) the period for which high levels are retained. These observations are supported by a statistical analysis of the water level and earthquake frequency for the Lake Mead⁶, Koyna,

TABLE 1 Koyna events of magnitude ≥ 4 since 1969

Date	Magnitude	Date	Magnitude
January 21, 1969	4.1	September 25, 1970	4.6
February 13, 1969	4.2	September 26, 1970	4.6
March 7, 1969	4.4	January 23, 1971	4.2
June 3, 1969	4.2	February 14, 1971	4.0
June 27, 1969	4.5	August 10, 1971	4.0
July 22, 1969	4.0	August 10, 1971	4.3
November 3, 1969	4.1	May 1, 1972	4.2
November 4, 1969	4.2	May 11, 1972	4.5
April 16, 1970	4.0	November 11, 1972	4.1
May 27, 1970	4.8	April 19, 1973	4.1
June 8, 1970	4.1	October 17, 1973	4.0
June 17, 1970	4.1	October 17, 1973	4.1
September 21, 1970	4.0	October 17, 1973	5.1
		October 24, 1973	4.6

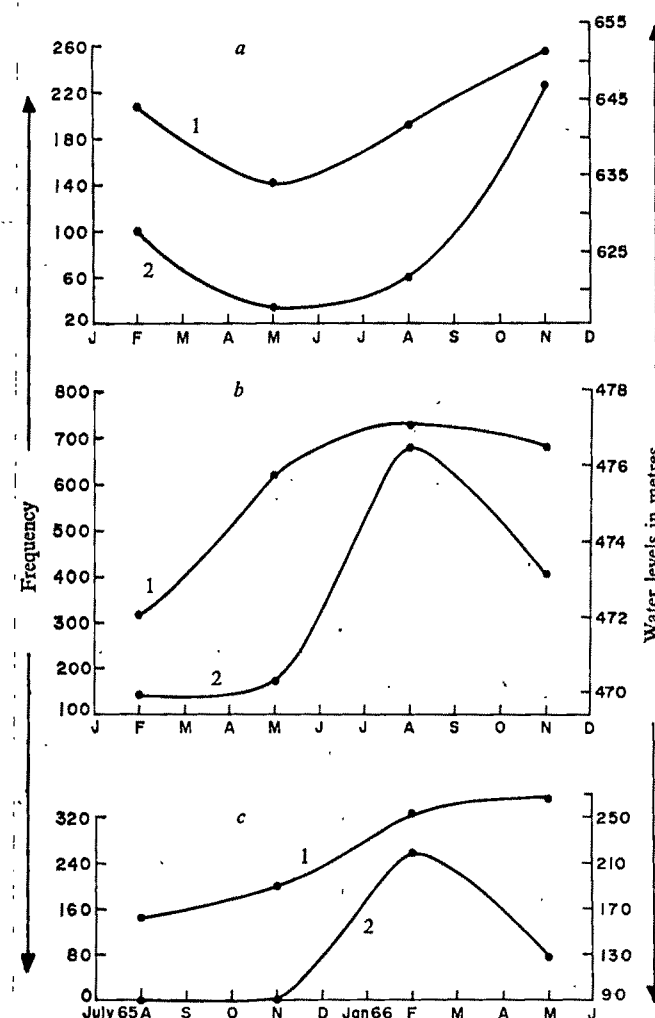


FIG. 1 Three-monthly average of water levels and total numbers of earthquakes for the same months at Koyna (a), Kariba (b) and Kremasta (c) for which the correlation coefficients are +0.93, +0.74 and +0.69, respectively. The periods include 1964 to 1968 for Koyna, 1959 to 1968 for Kariba and July 1965 to June 1966 for Kremasta. 1. Water level; 2, frequency.

Kariba and Kremasta reservoirs. In Fig. 1, for the latter three cases, water levels are given as averages over 3 months and the earthquake frequency as the total number of earthquakes registered in the same months for the whole of the period considered. The correlation between water level and frequency is readily apparent. Using other averages and monthly combinations, however, the correlation is less apparent and the high activity before and after the main shock greatly affects the frequency curve. Among the many examples of reservoirs with associated seismicity Koyna is the most outstanding; earthquakes occur whenever high levels are reached following rapid filling and are retained for long times. The highest water levels were maintained for longest⁷ during August to December 1967, corresponding well with the maximum seismic activity and the earthquake of magnitude 6.0 on December 10, 1967. The second highest level was reached during August to October 1965, which corresponds well with the second most conspicuous period of activity (during November 1965).

Reservoir levels in the Koyna Lake have been kept relatively much lower since December 10, 1967. No major earthquakes have occurred since October 1968 and this year water has again been allowed to accumulate in the reservoir, which was filled completely for the first time on August 15, 1973. The consequent high seismic activity included an earthquake of magnitude >5 and very strongly supports the idea of a persistent relationship between water level and earthquake activity.

The earthquake of October 17 is similar to that of September 13, 1967. The two earthquakes had comparable magnitudes, originated at the same place, had a similar foreshock-aftershock pattern and occurred after rapid filling of the reservoir to peak levels. Although it cannot be said with certainty that the present seismic activity heralds a strong earthquake in the Koyna region, there is a definite enhancement in the seismic activity which correlates with the increased reservoir levels.

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Gradient of caesium in the ocean

THE major alkaline metals are extremely uniformly distributed in oceanic water relative to other solutes. Caesium occurs in the ocean in such small traces, however, that precise analysis has in the past been difficult and its distribution in the sea has remained in doubt. Samples collected at seven depths in the North Pacific in 1970 were measured using a differential flame photometer, which indicated, that caesium concentrations in the open North Pacific vary by no more than about 2% between depths of 0 and 4,000 m (ref. 1). Unfortunately, this earlier analytical procedure, and others in use, are incapable of demonstrating the still smaller anom-

alies and concentration gradients that must exist in an ocean that continues to transport caesium from its terrestrial rocky sources to the seafloor.

Less is known about the long term steady state of progress of the natural non-radioactive nuclide ¹³³Cs through the ocean, than is known about the progress of the radioactive nuclides of caesium that have entered as nuclear fallout during the last decade. It would be reassuring to understand, for instance, how fast the surface layers of the ocean might dissipate the relatively larger amounts of ¹³⁷Cs that may enter as a consequence of the use of fissionable fuels.

Recently, analytical equipment specialized for traces of natural caesium was completed at the Scripps Institution² and tested with carefully collected North Pacific water samples. These measurements seem to demonstrate for the first time a significant difference between the caesium concentrations at the sea surface and in the deeper water. They also emphasise the uniformity of caesium concentrations in deep Pacific water masses, and the extreme similarity of caesium concentrations, normalised against salinity, at Pacific surface stations thousands of miles apart.

In essence, the new procedure is a computerised version of emission flame spectrometry, used after relatively large (2 l) samples have been concentrated by multiple extractions using the methods of Feldman and Rains³. Standard amounts (spikes) of caesium are added to alternate halves of each unknown and each reference sample, which are extracted in special gang tumblers⁴. A new form of sample changer which uses an automated distributing valve² burns the unknown spiked and unspiked concentrates, as well as the spiked and unspiked concentrates of 'reference standard seawater samples' in uniform succession. Burning of each of the four concentrates is done on six portions alternatively, and is programmed by a digital system tied into a desktop computer (Wang 600).

This allows standardising assays (on 10 ml organic concentrates containing 0.3 µg caesium) to be replicated with standard deviations < 0.3%. At present, the standard error of replicating the complete comparison between two seawater samples is somewhat more, about 0.5%. Two to eight replications are, however, made.

Figure 1 summarises concentrations and positions at six North Pacific stations. Caesium determinations are normalised to a salinity of 35.00 parts per thousand and the bars give the standard deviation of the mean of assays for each sample. In August 1973 at stations 1 and 2 small (4 l) samples were collected at depths of 0, 1,000, 2,000, and 4,000 m. At station 1, a large (50 l) poly carboy of surface water was collected for tentative reference. Large samples were also collected at stations C and D, about 2,000 miles apart. The normalised means of caesium concentration for these three large recent samples differed little, 0.0005 (0.2%), from a mean value of 0.2990 µg kg⁻¹.

The (large) 3-year-old samples, A and B, had concentrations departing from this mean less than might be expected from (wall) losses, and the two smaller surface samples, 1 and 2, differed only as predicted statistically.

Figure 1 indicates a downward decrease of natural concentrations of caesium in the open North Pacific Ocean. It is too early to discuss the differences apparent between 1,000 m and 4,000 m, but the mean of all (12) subsurface analyses of the six samples collected at 1,000 m or below is 0.295 µg kg⁻¹ and the difference between this and the surface mean is 0.004 µg kg⁻¹ (1.4%). This seems to be a significant measurement, of interest to oceanographers.

Caesium, of all the alkali metals, has the shortest mean residence in the ocean, about 0.9 m.y. (ref. 5). Concentrations in typical deep sea sediments, 0.9 p.p.m., (ref. 5), imply steady transport rates that are quite consistent with mid-water concentration gradients implied by the data summarised in Fig. 1. For example, if simple constant-coefficient diffusion

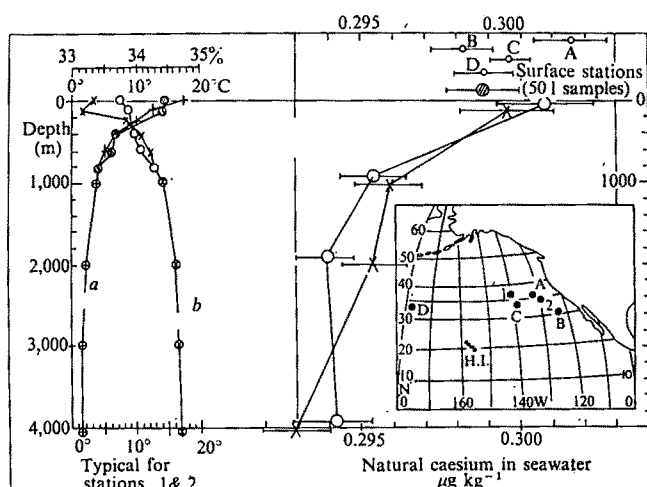


FIG. 1 Variations in natural caesium (^{137}Cs) concentrations in the North Pacific (normalised to salinity 35.00‰). The map shows positions of the stations sampled: A and B in 1970; C, D, 1, and 2 in summer 1973. Precision for caesium measurements had to be greatly advanced to make these comparisons. Tentatively a mean value of $0.299 \mu\text{g kg}^{-1}$ of Cs for North Pacific surface water was concluded. Deep water seems to have 1.4% less caesium. a, Temperature; b, salinity; open and hatched circles, deep station 1; +X, deep station 2.

through an ocean 'cap' 1,000 m thick is tentatively considered, a mean coefficient of 0.2 is required. Thinner 'caps' require correspondingly smaller diffusivities which range within values suggested by several other midwater studies⁶⁻⁷. Further assurances come from finding that the changing vertical concentration profiles⁸⁻⁹ of fallout radiocaesium, ^{137}Cs , are consistent with mixing rates approximately expressible by coefficient of these magnitudes, as are the changing concentrations of radioactive caesium observed in tissues of oceanic fish during the past decade¹⁰.

The mean surface value of $0.299 \mu\text{g kg}^{-1}$ seems free from absolute bias to well within 1%. Studies of chemical blanks and the linearity of signals with concentrations are, however, being further investigated to refine this absolute measurement. It is now evident, from comparisons of 1970 samples A and B with recent samples C, D and 1, that the large storage losses previously feared may be overcome, although to what extent is still uncertain. It is, nevertheless, evident that investigations of oceanic caesium should be referred to large contemporary samples of seawater of well established salinities, preferably deep water samples.

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Change in the current regime in the Suez Canal after construction of Aswan High Dam

Morcos and Messieh¹ attempt to confirm conclusions by Morcos², exemplified by the statement "Comparison with older data from the Canal in September 1924, September 1933, September 1954 and September 1964 showed that a complete reversal of the current regime at that time [September 1966] had taken place."

In their recent letter¹ other observations of 1966 were presented which, they claimed, 'confirm that the southward current which has hitherto occurred in the Canal every summer, did not occur in the summer of 1966 and that the current remained northward during the whole year.'

It is difficult to see how such a statement could be maintained in face of El Sabh's^{3,4} observations of September 1966. In Morcos and Messieh's words describing El Sabh's observations "The northern part of this section, unlike Morcos's section, shows that the northern part of the canal was filled with Mediterranean water." Noting that this northern part is more than 70 km long, Morcos and Messieh's self-contradiction becomes apparent, as the Mediterranean water could not conceivably penetrate that far south, with a northgoing current prevailing all year round.

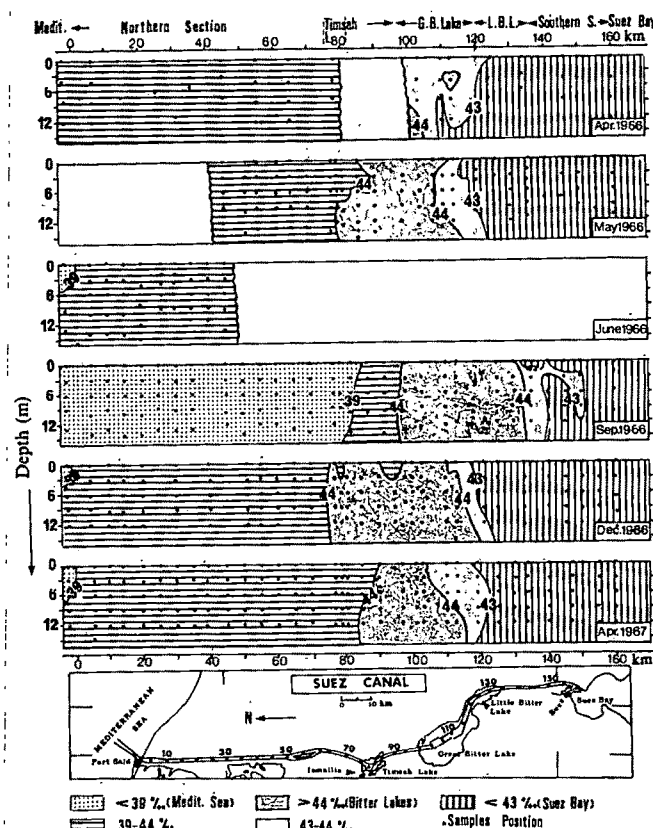


FIG. 1 Salinity with depth section of the Suez Canal in 1966, showing positions of stations and depths at which samples were taken.

The explanation is that Morcos's observations on September 29–30, 1966 were made after the normal September situation has ceased and the northgoing current resumed flowing.

As to their assertion that the net current in the southern part of the Canal was also northgoing, based on observations that the Great Bitter Lake (GBL) water did not reach the Suez Bay, this is also a conclusion not warranted by the evidence advanced.

For the GBL water to reach the Suez Bay, it has first to fill the Little Bitter Lake, then fill the southern Canal and only then can it be detected in the Suez Bay. But it can move southwards to the Little Bitter Lake, the southern part of the Canal and even into the Suez Bay. The southern limit of GBL water will depend upon the net speed and duration of the motion. Comparing the September 1966 observations with those in May and December 1966 (Fig. 1) the GBL water has moved south to fill Little Bitter Lakes in September. Because the cross-sectional area of the Little Bitter Lake is five times the mean cross section of the Canal proper, the filling of this lake (~15 km long) is equivalent to filling about 75 km of the Canal proper. It is admitted that the southern motion was not as strong in 1966 as previously observed, but it would be a mistake to insist that it did not occur.

Our conclusion is that the usual reversal of the Suez Canal regime occurred during 1966, as it did in previous years. The magnitude of the reversal in that year was not as great as previously observed, and further observations are necessary to establish the magnitude of the influence of the cessation of the Nile flood on that reversal, but certainly it did not stop it.

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DR MORCOS replies: Hassan and El Sabh compare one season with another by using their five sections of 1966. My approach is to study the seasonal cycle of 1966 in the light of the seasonal observations made during the preceding years, with a view to detecting any change that could have taken place after the construction of the Aswan High Dam.

Between 1933 and 1937, the scientists of the Suez Canal Company conducted an extensive 'quantitative' study of the water regime of the canal by registering the mean water level and currents using five tide gauges and two continuous recording current meters north and south of the Great Bitter Lake. Baussan¹ concluded that between the northward current in winter and the southward current in late summer, there is a transitional period during which the current is only reversed in the northern canal. Thus

water flows from the Mediterranean to the Bitter Lakes while the current in the southern canal continues to flow northward from the Red Sea to the Bitter Lakes. Examples given by Baussan were July–August 1935, June, July, August 1936, and June 1937. In these months the water flowed from both ends of the canal towards the Bitter Lakes. This is the most likely regime since, according to Miller and Munns², compensation must be made for the high evaporation from the Bitter Lakes during this season. Thus, Hassan and El Sabh's observation that Mediterranean waters penetrated 70 km into the northern canal does not necessarily imply that the current throughout the canal (the net out-flow) was southwards.

My studies³ of current measurements in the promontory of Kabret, between the two basins of the Bitter Lakes, indicate that the resultant currents are much weaker and less variable in magnitude and direction, and may even show a sign opposite to that of the stronger currents in the southern canal. The water mass of the Great Bitter Lake (GBL) increases in volume and salinity in summer because of high evaporation and slow currents over the salt bed^{4,5}. This is most easily observed in transitional periods when water flows from both ends towards the Bitter Lakes as a result of the long residence time over the salt bed. The Little Bitter Lake (LBL) is an integral part of the Bitter Lakes Basin and in the absence of a strong northward current is filled by the water mass of the GBL as a result of the expansion of this water mass in volume and its increase in salinity. This is enhanced by diffusion and turbulence processes and should not be taken as evidence of a southward current in the canal, as suggested by Hassan and El Sabh. A similar distribution, showing that the LBL filled with GBL water mass, was observed in August and November 1931, July, August, October and November 1933, June and July 1935 and June 1955. These are months with transitional characteristics or even a weak northward current.

In the various parts of the Suez Canal, currents change direction over periods of hours and days as a result of tidal and non-tidal factors. The main seasonal criterion, however, is the net outflow from the canal, which quantitatively defines the current regime. This is probably an appropriate expression of the final resultant of the complex system of currents in the canal.

Continuous recording of currents at two points in the southern and northern canals has quantitatively demonstrated a net outflow into the Mediterranean Sea during most of the year and a net outflow into the Red Sea during late summer. For example, in 1935 there was a net outflow of $5,250 \times 10^6 \text{ m}^3$ into the Mediterranean during the first period, and of $150 \times 10^6 \text{ m}^3$ into the Red Sea during the second period⁶. Passing through the Great Bitter Lake, this latter outflow greatly increases the salinity in the Suez Bay during August and September every year. It has been demonstrated that such an increase in salinity did not take place at all during 1966⁷ and it can be safely stated that the net outflow from the canal into the Red Sea previously observed every late summer, did not occur in the summer of 1966.

Finally, Hassan and El Sabh admit that an unusual phenomenon occurred in the current regime in the Suez Canal during 1966 and that "further observations are necessary to establish the magnitude of the influence of the cessation of the Nile flood on the reversal of the current". In their view, this unusual phenomenon is only a change in the magnitude of the reversal, whereas it is, according to the evidence present here, a fundamental change in the seasonal pattern of the current regime. My conclusion is that the northward current regime in 1966 was followed by a transitional period with water flowing from both ends to the Bitter Lakes. By mid September the current had

regained its northward direction without being reversed to the south.

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Time-dependent viscosity effects measured on an impact microviscometer

THE viscosity of a fluid has been shown by conventional viscometers to rise approximately exponentially with pressure. Such viscometers always require a long period of time to build up the pressure on the fluid, typically an hour or more to reach pressures common in elastohydrodynamic contacts. Since a typical residence time for a fluid in a contact would be less than 0.01 s, Fein has suggested that fluids might not reach equilibrium viscosities instantaneously¹. Until now there has been no direct experimental evidence to support this suggestion.

We have already published some viscosity-pressure data measured on an impact microviscometer², and Hutton and Phillips have found the viscosity-pressure characteristics of one of the fluids using a Couette viscometer³. A large discrepancy is seen when the two sets of results are compared, except at the very low pressure end. Above 60 MN m⁻² the discrepancy increases steadily until, at the highest pressure reached, there is a difference of almost four decades in viscosity. We suggest here a reason for this enormous discrepancy.

The technique used to determine viscosity has been described². In brief, the elastohydrodynamic film of liquid trapped between a flat surface and a loaded ball is measured using a new interferometric technique. From the variation of film thickness with position and time the viscosity can be calculated using Reynolds equation in polar co-ordinates.

The pressure field is found from the elastic deformation of the surfaces. The shear stress τ , given by $\tau = (1/2)h\delta p/\delta r$, is quite large, reaching 50 MN m⁻². These stresses might cause the fluid to be non-Newtonian (K. L. Johnson, personal communication) thus explaining the difference between our results and those of ref. 3. In the dimple the shear stresses rose to a maximum at about $r = 0.3R$ (where R is the radius of the entrainment measured from the centre), and fell to zero at the centre where $\delta p/\delta r = 0$ and at the edge where h is small.

If the critical shear stress, above which the liquid is non-Newtonian is less than the maximum shear stress, the viscosity measurements would only be correct near the centre and edge of the entrainment, but would be too low in the intermediate position. Typical viscosity measurements with the impact microviscometer show very little increase of viscosity with pressure in the high pressure regions, the viscosity measured at $0.3R$ is very much the same as the viscosity near the centre. Taking into account a shear stress

effect lowering the effective viscosity would mean that the 'true' viscosity is considerably larger than the observed values in the intermediate region. This implies that 'true' viscosity reaches a peak as the pressure is increased and then falls at the extreme high pressures, which is most unlikely. Thus it is reasonable to conclude that the shear stresses here are not high enough to cause non-Newtonian behaviour in the fluid.

The next factor to be considered was whether there could be any time dependence of the viscosity on the pressure step imposed by the ball. In previous measurements there appeared to be no systematic variation of results taken at successive intervals of time as the fluid leaked out of the entrainment. It was realised however, that compared with the time intervals of the measurements these results were taken a long time after the pressure step was applied.

The viscosity of one fluid was therefore measured using a ciné-camera at 400 frames s⁻¹ to obtain viscosity measurements at times from 0.01 to 5 s after the imposed pressure step. Some results were also taken on a new apparatus which, by using a plate made of sapphire instead of glass, tolerates much higher pressures. Because of the higher pressure, the fluid took much longer to flow and results could be obtained at times of up to 200 s after the pressure step. The results for the chosen fluid, a paraffinic cylinder stock B P 10.65, are shown in Fig. 1. There is a definite time delay of the viscosity rise after a pressure step. At 800 MN m⁻² the viscosity varies from 3×10^4 N s m⁻² after 0.015 s, to 3×10^8 N s m⁻² after 105 s.

The polyphenyl ether tested in the earlier experiments^{2,3} was then measured at the longest possible time after the pressure step to see if there would then be a better correlation with the results of Hutton and Phillips. The new results, together with the earlier ones^{2,3} are shown in Fig. 2. The new results show a better correlation although the curves still diverge at a pressure of 150 MN m⁻². This is to be expected since the technique of Hutton and Phillips² requires a considerably longer time to reach this pressure than the 130 s in our experiments, so the discrepancy in the results can probably be attributed to time-dependence effects. Also included in Fig. 2 for comparison is a viscosity-pressure characteristic obtained for polyphenyl ether 5P4E⁴. This was derived from traction data on the fluid as it passed through a rolling/sliding elastohydrodynamic contact at 99° C and therefore refers to time scales of much less than

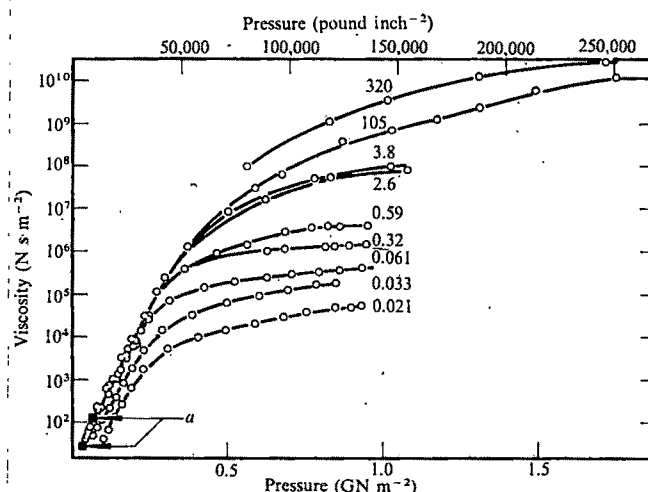


Fig. 1 Variation of viscosity with pressure of fluid BP 10.65 paraffinic cylinder stock, showing time delay of viscosity increase after a pressure step. The figures following each curve indicate the amount of time which has elapsed (s) since the first photograph (see text). a, Viscosity measured on a conventional viscometer.

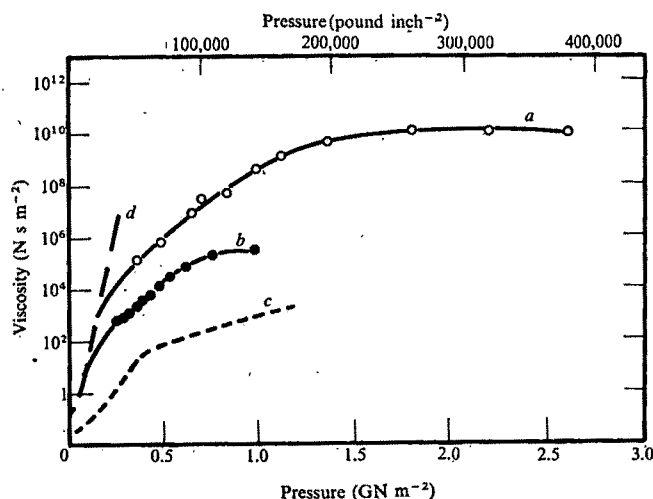


Fig. 2 A comparison between the new results and results obtained in earlier experiments. *a*, Fluid 5P4E at 23° C after 130 s; *b*, fluid 5P4E at 23° C after 5 s; *c*, fluid 5P4E at 99° C, derived from traction data⁴; *d*, fluid 5P4E measured on a Couette viscometer³.

our results in Fig. 2. No direct comparison can, therefore, be made but it is interesting to note that the same general behaviour is found.

In conclusion, it seems that time-dependent variation of viscosity following a pressure step, first proposed by Fein¹, has been experimentally established. This will have a profound effect on estimates of viscosity inside elastohydrodynamic contacts. We are now investigating the time dependence for other fluids.

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Surface structures of gibbsite goethite and phosphated goethite

INFRARED studies of surface hydroxyls on solids of high area have been limited to almost anhydrous materials prepared at elevated temperatures. These studies have made an important contribution to the understanding of surface catalysis, but have little relevance to adsorption processes that occur on moist surfaces at ambient temperatures. Such processes contribute to a wide range of everyday phenomena, including the retention of fertilisers and pesticides in soils.

Here we establish that the surfaces of the crystalline hydroxides gibbsite (γ -Al(OH)₃) and goethite (α -FeOOH), both common soil components, are accessible to infrared study, and that their surface structures are well defined and closely related to their bulk structures. The observation of

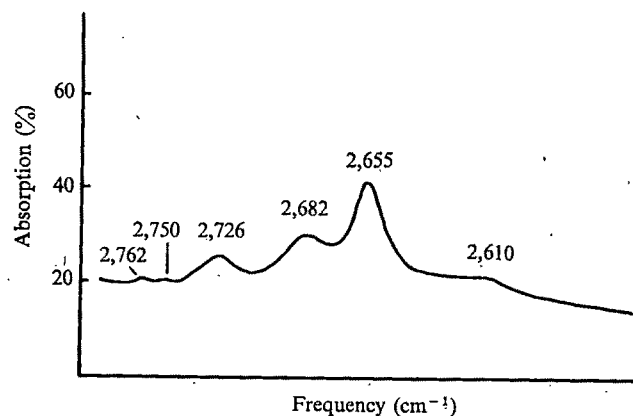


Fig. 1 Infrared absorption of surface Al-OD groups on the (001) face of gibbsite in the form of a polycrystalline film (6.2 mg cm⁻²). Weak bands at 2,762 and 2,750 cm⁻¹ may arise from Si-OD of adsorbed silicic acid; the band at 2,610 cm⁻¹ is due to bulk OD.

these surface hydroxyls opens the way to the study of their involvement in surface reactions.

Electron microscope analysis shows the synthetic gibbsite¹ was in the form of thin hexagonal plates of diameter about 250 nm and thickness 9 nm. Their surface consisted principally of (001) faces with a calculated area of 96 m² g⁻¹, whereas edge faces represented only 8 m² g⁻¹. On evaporation from suspension, this material formed excellent self-supporting films with the crystal plates parallel to the film plane.

The infrared spectrum of this gibbsite is dominated in the 3,000 to 4,000 cm⁻¹ region by absorption due to the stretching vibrations of bulk hydroxyl groups, which give bands at 3,622 cm⁻¹ and 3,529 cm⁻¹ polarised in the (001) plane, and a third at 3,460 cm⁻¹ polarised perpendicularly to that plane. Gibbsite consists of infinite planes of close-packed hydroxyl groups parallel to (001), the planes being bound together in pairs by aluminium ions to form a layer structure. Thus the perpendicular 3,460 cm⁻¹ band can be correlated with hydrogen bonds between layers (2.82 to 2.87 Å long²) and the 3,622 cm⁻¹ and 3,529 cm⁻¹ bands correspond to longer hydrogen bonds between hydroxyls within the same plane (3.29 Å and 3.13 Å long according to ref. 2; a longer in-plane OH-OH separation of about 3.38 Å probably involves no interaction).

Absorption by these bulk hydroxyls largely obscures the absorption bands of surface Al-OH groups, but a brief (10 s) treatment of a gibbsite film with D₂O followed by evacuation revealed surface Al-OD groups absorbing at 2,726 cm⁻¹, 2,682 cm⁻¹ and 2,655 cm⁻¹ (Fig. 1), which were lost again immediately on exposing the films to water vapour. The corresponding frequencies of surface Al-OH groups would be 3,690 cm⁻¹, 3,629 cm⁻¹ and 3,588 cm⁻¹ but only the first can be seen in evacuated films because of the strong absorption by bulk hydroxyl groups. All three surface absorption bands are polarized in the plane of the layers, so the OH groups that were involved in interlayer bonding within the crystal (absorbing at 3,460 cm⁻¹) must link OH groups within the surface plane, and probably absorb at 3,690 cm⁻¹. This weak interaction leads to a lengthening of other in-plane OH-OH separations, so that hydroxyls that absorb at 3,529 cm⁻¹ and 3,622 cm⁻¹ within the crystal absorb at 3,588 cm⁻¹ and 3,629 cm⁻¹ on the surface.

The synthetic goethite used was prepared as was specimen 1(c) of ref. 3, and, like that specimen, consisted of thin laths with the (100) faces predominant, typically measuring 150 nm in the [001] direction, 30 nm wide and 10 nm thick. The calculated area of the (100) faces is 45 m² g⁻¹, with edge planes contributing about 15 m² g⁻¹.

The goethite structure (Fig. 2) can be regarded as being built from strips of condensed Fe(O, OH) octahedra, each

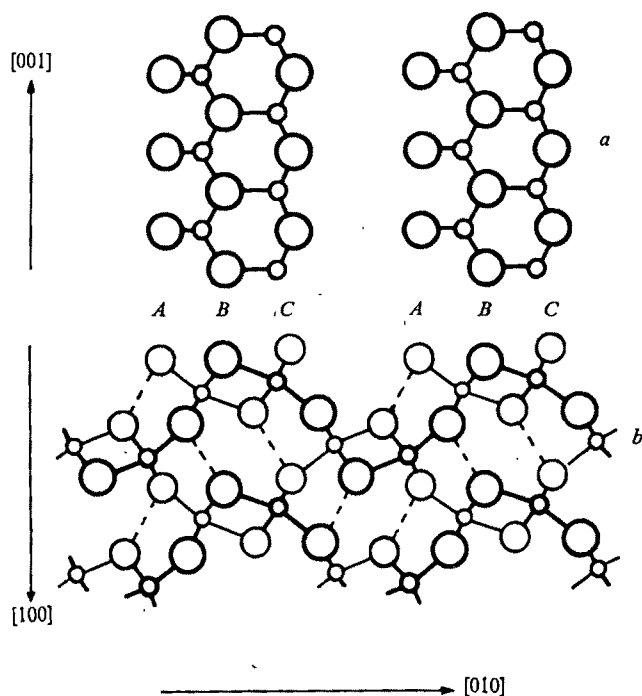


Fig. 2 Plan (a) and section (b) of the (100) face of goethite (after Bragg and Claringbull⁶).

strip being two octahedra wide. The strips, which are seen in plan in Fig. 2a, and in section in Fig. 2b, share O^{2-} ions along their edges to give an open structure in which the gaps between the strips are bridged by hydrogen bonds (dashes in Fig. 2b) involving OH groups shared between three Fe^{3+} ions. These bulk hydroxyls absorb at $3,145\text{ cm}^{-1}$. The same hydroxyls (labelled B in Fig. 2) are also exposed on the (100) surface together with two new types of hydroxyls (labelled A and C) which arise from protonation of the oxide ions along the edges of the strips. Of these surface hydroxyls,

types B and C cannot form hydrogen bonds within the surface, since all adjacent hydroxyls are coordinated to a common Fe^{3+} ion, but type A hydroxyls, each coordinated to only one Fe^{3+} , can form bonds 3 \AA long with each other.

In the spectra of films of goethite examined *in vacuo* (Fig. 3a) type A hydroxyls absorb at $3,486\text{ cm}^{-1}$ and the non-bonded hydroxyls absorb at $3,660\text{ cm}^{-1}$. These surface hydroxyls are readily converted to OD, absorbing at $2,700\text{ cm}^{-1}$ and $2,581\text{ cm}^{-1}$, by brief (1 min) treatment with D_2O , and they interact with adsorbed water when the films are exposed to air, as shown by broadening and displacement of the absorption bands.

Goethite that had been treated with $200\text{ }\mu\text{mol per g H}_3\text{PO}_4$ or NaH_2PO_4 before preparing films showed no absorption due to type A hydroxyls (Fig. 3b), thus confirming the hypothesis of Atkinson *et al.*⁴ that phosphate replaces these hydroxyls, forming bridges between adjacent Fe^{3+} . The spectra also demonstrate an interaction between phosphate and B or C type hydroxyls, resulting in new bands appearing at $3,671\text{ cm}^{-1}$ and $3,646\text{ cm}^{-1}$. This could represent the effect of a hydrogen bond between surface $(FeO)_2PO\cdot OH$ groups, acting as proton donors, and type C hydroxyls. These and other reactions of surface hydroxyl groups on hydroxides are being actively investigated.

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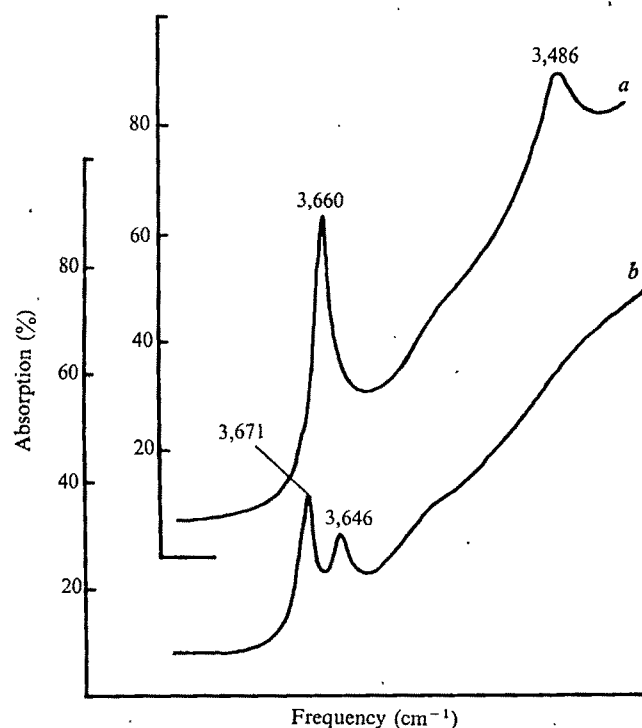


Fig. 3 Infrared absorption of surface OH on: a, Goethite; b, phosphated goethite. Both in the form of polycrystalline deposits (5.4 mg cm^{-2}).

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Gravitational charge, hadron hydrodynamics and Gödelised hadrons

I discuss here the consistency of approach which is not immediately apparent when current approaches to particle physics are compared. The parton model, *f* gravity and the hydrodynamical model of multiple particle production in high energy hadron collisions complement each other in remarkable ways and point to a useful general formalism for particle physics problems in such diverse applications as mass spectra through collisions to the first picoseconds of the Universe according to the big-bang theory.

Motz has obtained an expression for gravitational "charge" in the interior of fundamental particles based on the Weyl theory of gauge invariance¹ and on the Schwinger (actually Bohr-Sommerfeld) quantisation procedure² which implies a value for the gravitational constant of $\sim 1.8 \times 10^{31}$ (c.g.s. units) within a proton (or any hadron). Bakesigaki and Inomata³ have derived a value of $G \sim 1.2 \times 10^{32}$ from the strong *f*-gravity theory⁴ and a model of hadrons as de Sitter spacetimes possessing rotational degrees of freedom (relativistic "rotators")⁵ by obtaining a mass formula for hadrons

under the assumption of conformal $SO(4, 2)$ symmetry (invariance) being broken into the $SO(4, 1)$ symmetry group, the masses then corresponding to the energy levels specified by the angular momentum quantum number J .

What other sort of relativistic rotator is consistent with the existence of such large values of the gravitational constant under a gravitational charge hypothesis applied to hadronic matter (strongly interacting fundamental particles)? The simplest models should always be considered first, so I will look for classical phenomena which may still be useful for qualitative description in the quantum domain of the hadrons. The Landau hydrodynamical-thermodynamical fluid description of multiparticle production^{6,7} has recently been reconsidered with some success^{8,9}. So it seems useful to seek a fluid description, and just look at the proton as an infinitely bootstrapped¹⁰ hadron ground state. Another semiclassical approach has involved the use of the cosmological constant in the Einstein equations as the inverse square Compton wavelength of the f^2 -meson and interpreting the meson spectrum as that of an oscillator, thereby coming within half a pion mass of the proton's mass for the ground state of a hadron¹¹. I note also that the parton model of hadrons containing an almost infinite number of almost light-like particles¹² lends itself easily to a statistical-fluid description of the hadron in terms of its constituents. So I conclude that it is useful to examine a fluid description of the hadrons in which the cosmological constant also may have meaning.

One possibility is Gödel's universe¹³. To examine this I work with Gödel's spacetime line element in the form $ds^2 = (dx_0 + \exp(ax_1) dx_2)^2 - dx_1^2 - (\frac{1}{2}) \exp(2ax_1) dx_2^2 - dx_3^2$, where a is related to the rotation velocity w and the speed of light, c by $w = (ac/2)^{1/2}$ (ref. 14). The Einstein field equations for such a line element then imply that the density u and pressure p of the matter are related by $p = u = a^2$ (in geometrised units), implying unusual properties for such matter¹⁵. An alternative interpretation of the Gödel solution would constrain the fluid to $p = 0$, $u = -\Lambda/4\pi$, where Λ is the cosmological constant, and $\Lambda = -a^2/2$ (ref. 14).

So there are two possible approaches. In the first case, one fixes the field of the proton near its surface from application of the Kerr-Newman metric in an f -gravity form¹⁶, thereby fixing the curvature for the density u and then matching that curvature *a priori* to $u = a^2$ in the Gödel solution. The second case would involve self-consistent use of the relation $u = a^2$ entirely within the Gödel solution but that would make no reference to f gravity and allow no connection of possible exterior and interior solutions to the field equations $G_{ab} = -k T_{ab}$ for a fluid hadron, which is a part of the motivation for this work. I use the former.

Let the rotation w be given by the usual relation of angular momentum L to moment of inertia I ($w = L/I$) and let $I = Q_p MR^2$ where M is the particle mass, R is its radius and Q_p is the gravitational charge. Inserting the parameters for the proton of $M = 1.6 \times 10^{-24} g_m$, $L = \hbar/2$ ($\hbar =$ Dirac's constant), and $R \sim 10^{-13}$ cm, using the Kerr-Newman curvature from the components of the Riemann tensor in the equatorial plane ($R_{\theta\phi\theta\phi} \sim GM/c^2 r^3$), $u \sim 1.1 \times 10^{-12}$ cm², and further augmenting the gravitational effect of the mass implied by the ultrarelativistic 'equation of state' $p = u$ (that is, doubling the mass), we find $Q_p \sim 3 \times 10^{17}$. Applying the charge relation of Motz² ($Q = G^{1/2}$) we find $G = Q_p^2 \sim 9 \times 10^{34}$. This is a larger value of G than found by others^{1,2} but it does augment their impression of extremely large values of G within hadrons.

The values may be brought into agreement by detailed consideration of the charge distribution in the particle¹⁷ or its mass distribution (these distributions being possibly of equal value here because an application of the parton model with a vector gluon interaction between partons has shown

that the two distributions are related and may be identical¹⁸), by perhaps allowing a density distribution $u \sim u_0 \exp(-x_1/c)/x_1$, where l is a scale length. That is not unreasonable since it is the coupling of the field and its source which produces gravitation and this source distribution has the structure of the Yukawa field. Peak's consideration of the relation of strong gravity and the Yukawa potential, indeed, derives an equation which implies such a form of u (ref. 19). Such a distribution yields a decreased requirement on Q_p in the moment of inertia, implying a value of $G \sim 3 \times 10^{32}$. I have found that the Einstein tensor for the inhomogeneous Gödel model calculated from the distribution $u_0 \exp(-x_1/c)/x_1$, with $a = a(x_1)$, contains non-zero G_{12} terms corresponding to stresses in the matter. These determine Maclaurin spheroids which may be matched to the quadrupole moment and angular momentum relation in the Kerr-Newman solution and its axis-symmetric null surfaces.

Using the cosmological constant instead gives the same results. The only problem, that of consistency with present work in particle physics, involves the $p = 0$ equation, but hadron models with such a constraint are under construction by a group at Massachusetts Institute of Technology and it has been shown that a hadron gas under conditions of compaction of the early Universe similar to that of the maximum temperature ($T_m = 160$ MeV) hydrodynamical model²⁰ may obey an equation of state such that u is so much greater than p that p may be set equal to zero by comparison with u (ref. 21).

The above consideration is worth noting because there now exists the formalism of the tilted universe in cosmology²² which is immediately adaptable to the $f - g$ gravity theory by adding the coupling terms in the field equations to the cosmological equations⁴, with or without cosmological constant (with or without altered constraints on the equation of state such as $p = 0$). But the tilted frames are specified by the same coordinate transformations which specify the infinite momentum frames of the parton model¹²; so either way, a complete formalism now exists to do the complete physics of the first picoseconds of the big-bang origin of the universe, including gravitation, hydrodynamics and thermodynamics, and particle physics under the constraints of completely and consistently specified models.

There is, in conclusion, a remarkable consistency in these new gravitationally/hydrodynamically oriented approaches to the structure of the fundamental particles and a much greater range of applicability (from hadrons to 3C295) than has been attempted.

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BIOLOGICAL SCIENCES

Specific recognition of the isolated R17 replicase initiator region by R17 coat protein

Coat proteins from the RNA bacteriophages of groups I (R17, MS2, f2) and III (Q β) specifically bind to their homologous RNAs and serve as translational repressors of replicase synthesis¹. In the case of R17, a fragment of the genome which is protected by coat protein from T₁ RNase digestion² contains not only the ribosome binding site of the replicase cistron³, but also the preceding intercistronic region and 23 nucleotides (including the terminator triplets) from the coat gene⁴. Physical studies described in the previous papers^{5,6} suggest that the structure of this isolated R17 fragment consists of two hairpin loops joined by a single-stranded region; the coat protein binds in such a way as to alter the behaviour of only one of these helical regions⁵, that which contains the initiation site for the replicase gene. Here I show that this portion of the fragment alone is sufficient for recognition by the coat protein.

Isolated protein synthesis initiator regions from R17 RNA⁷ provide a convenient source of the 3' terminal one-third of the coat protein-protected fragment (helix *b* of Fig. 1, ref. 5). Such ribosome binding site preparations contain a mixture of the beginnings of the three R17 genes, each present in several size variants as illustrated in Fig. 1.

Table 1 and Fig. 2 show that the replicase initiator region can be both selectively and efficiently bound to Millipore filters by R17 coat protein. If the mixture of coat protein

A protein

AU · UC · UAG · GAG · GUU · UGA · CCU · AUG · CGA · GCU · UUU · AGU
 Coat

AGAG(c)C · UCA · ACC · GGG · CUU · UGA · AGC · AUG · GCU · UCU · AAC · UUU

Replicase

AA · ACA · UGA · GGA · UUA · CCC · AUG · UCG · AAG · ACA · ACA · AAG

Fig. 1 Oligonucleotide sequences of the isolated R17 initiator fragments⁷. Bold type indicates that portion present in the major size variant from each site as judged by homochromatography³ and by the relative yields of component T₁ oligonucleotides (see Fig. 2a).

and ribosome binding sites is treated with T₁ RNase before filtration, only those oligonucleotides which are at least partially contained in helix *b* (Fig. 1, ref. 5) are protected. Interestingly, Q β coat protein shows some affinity for the R17 replicase initiator region suggesting that it recognises a structurally similar site in its own RNA; the fact that the binding is less efficient and abolished by T₁ RNase probably explains why no protection of whole R17 RNA by Q β coat protein was previously observed².

The capacity of R17 coat protein to recognise the replicase

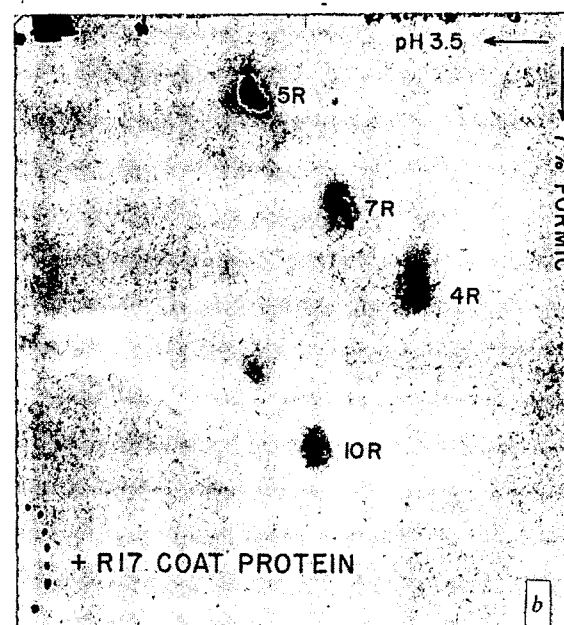
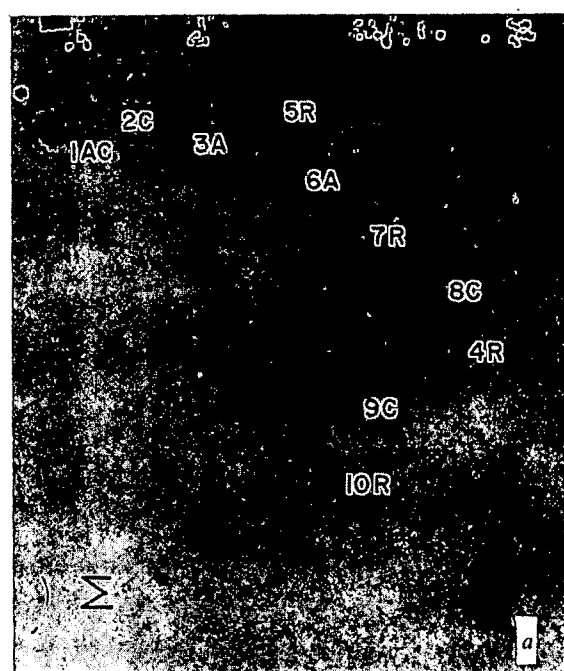


Fig. 2 T₁ RNase fingerprints of R17 initiator fragments (a) before incubation with, and (b) trapped on Millipore filter by R17 coat protein. Data is from experiment 1 of Table 1. Spots were identified by subsequent analysis as: 5R-AUUACCCAUG, 7R-AAACAUG, 4R-ACAACAAAG and 10R-UCG from the replicase site; 6A-ACCUAUG, 3A-AUCCUAG, and 1AC-UUUG from the A site; 9C-CAUG, 2C-CUUCUAACUUU, and 1AC-UUUG from the coat protein initiator region.

TABLE 1 Binding of initiator fragments by coat protein

	Aliquot of initiator fragment preparation	c.p.m. per $^{32}\text{PO}_4$ +R17 coat	+Q β coat	+R17 coat +T ₁ RNase
Experiment 1				
5R	112	152	67	
7R	59	97	45	
4R		44	15	
6A	136	11	27	
3A	57	4	15	
9C	274	13	27	
2C	73	1.6	9	
1AC	378	17	53	
Ratio of sites (replicase: A: coat)	1: 1.2: 2.5	1: 0.07: 0.09	1: 0.40: 0.40	
% Replicase site bound		30	11	
Experiment 2				
5R	207	59	18	20
7R	145	51	6	18
4R		17	6	—
10R		53	20	5
6A	215	—	6	—
3A	121	—	—	—
9C	563	—	9	—
2C	164	—	3	—
1AC		—	—	—
Ratio of sites (replicase: A: coat)	1: 1: 2.7	1: < 0.05: < 0.08	1: 0.3: 0.5	
% Replicase site bound		24	7	

R17 protein synthesis initiator regions were prepared by ribosome protection, isolated and characterised as described in ref. 7 and Fig. 2a. Reaction mixtures contained in 60 μl of TMK buffer (0.1 M Tris-HCl, pH 7.5; 0.01 M Mg acetate; 0.08 M KCl); 32 μg of isolated R17 ribosome binding sites (of which only 1–2% are the radioactive sites, the remainder being rRNA and tRNA fragments⁷) and 7.6 μg freshly prepared R17 or Q β coat protein⁸, or bovine serum albumin (BSA). After 10 min at 0° C, T₁ RNase at 0.1 mg ml⁻¹ was added as indicated and incubation continued for 30 min at 25° C. Samples were filtered through HA Millipore filters (24 mm) and the RNA extracted as described in ref. 2. Final preparations were analysed by two-dimensional T₁ fingerprints (Fig. 2b) and the above oligonucleotides counted and analysed. In both experiments, controls with BSA rather than coat protein, gave no detectible spots on the fingerprints. The oligonucleotides are identified in Fig. 2. Ratios of the sites were calculated from the relative yields of the underlined oligonucleotides, which contain the three initiator AUGs. The % replicase site bound was calculated from the fraction of replicase sites in the counts originally trapped on the filter. Spots containing less than 20 c.p.m. total were not detected and are indicated by —; spots were not counted where no value appears.

TABLE 2 Rebinding of coat protein-protected fragments

	Addition at		Filter at
	0 min	10 min	20 min
59-nucleotide fragment total, 22,650	Coat	—	5,800
	Coat	T ₁ RNase	5,100
	T ₁ RNase	Coat	108
	—	—	47
29-nucleotide fragment total, 10,680	Coat	—	2802
	Coat	T ₁ RNase	2616
	T ₁ RNase	Coat	60
	—	—	25

The 59- and 29-nucleotide fragments were prepared as described in ref. 5; after elution from the gel they were characterized by T₁ fingerprint analysis to be approximately 90% pure. Rebinding reactions were performed in 50 μl of TMK buffer at 25° C containing (as indicated): 3.8 μg freshly prepared R17 coat protein⁸, 1.4 μg of the 59-nucleotide or 0.7 μg of the 29-nucleotide fragment (a 4:1 molar ratio of coat protein to RNA), and 0.05 mg ml⁻¹ T₁ RNase. After dilution with 1 ml of TMK buffer, the samples were filtered with two 1 ml rinses through HA Millipore filters and counted in toluene scintillation fluid.

initiator region, as opposed to the 59-nucleotide RNA segment studied in refs 5 and 6, can also be examined directly since a T₁ digest of the coat protein R17-RNA complex yields both fragments (see Fig. 1, ref. 5). Table 2 shows that the 29- and 59-nucleotide fragments are retained on a Millipore filter by coat protein with comparable efficiency.

Thus it may be concluded that the 23 nucleotides at the

3' terminus of the 59-nucleotide fragment isolated from the R17 RNA-coat protein complex are sufficient for recognition by the protein. As originally suggested by Bernardi and Spahr², helix *a* (the coat cistron terminator region) most likely remains attached because of its great stability and resistance to RNase.

Must the R17 replicase initiator region assume a helical form to be recognised by coat protein? Its component T₁ oligonucleotides, which contain the sequence from the turn of hairpin *b* intact in oligonucleotide 5R (Fig. 1), are not efficiently rebound (Table 2); and studies presented in accompanying papers^{5,6} indicate that helix is formed by this portion of the 59-nucleotide fragment under physiological conditions. Formation of a secondary structure which sequesters the initiator AUG could explain the inability of *E. coli* ribosomes to recognise the isolated replicase initiation site efficiently⁷. In the intact R17 RNA, on the other hand, the region may well assume one of several alternative configurations⁸: an extended form, in which the initiator AUG may become available for ribosome binding after translation of an early portion of the coat gene; or the helix, which is subject to coat protein recognition and translational repression.

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High resolution proton NMR study of an isolated fragment of R17 bacteriophage mRNA

HIGH resolution nuclear magnetic resonance (NMR) spectra of the hydrogen-bonded protons in helical regions of nucleic acids are an informative monitor of the base pairing¹. Studies of about 10 purified tRNAs and of model systems have shown that the ring NH protons of uracil and guanine, when involved in Watson-Crick base pairing, give resonances between -15 and -11 p.p.m. downfield from DSS^{1,2}. Since there is only one such proton in each base pair, the intensity in this spectral region is a direct measure of the number of base pairs. Simple rules have been derived to predict the NMR spectra originating from these protons on the basis of ring currents from the adjacent bases and for tRNA the comparison between predicted and observed spectra has strongly supported the clover leaf model of base pairing. In addition, ring current shifts from bases adjacent to the helical region provided information about the structure at the end of the helix. Here we apply this approach to the secondary structure of the R17 fragment discussed in the preceding paper³. We prepared 0.5 mg of the R17 fragment according to a somewhat modified method of Bernardi and Spahr⁴, and measured the NMR spectrum of a 25 μ l sample.

Figure 1 shows the 300 MHz high resolution proton NMR spectra of the fragment at 26° C and 40° C, obtained with a Varian HR300 spectrometer after accumulation for ~7 and ~13 h, respectively. These spectra, with resonances between -15 and -11 p.p.m., are similar to those obtained^{1,2} from other nucleic acid molecules of comparable size (for example, tRNAs) although these lines are broader. Assuming that the peak at either -11.6 p.p.m. or at -14.3 p.p.m. represents one proton, the spectrum at 26° C has a total intensity corresponding to ~16 protons, in agreement with the number of base pairs expected from the structure shown in Fig. 1 of the previous paper³. Note that GU does not, in our experience, contribute a resonance in the region between -15 and -11 p.p.m.⁵

Upon raising the temperature from 26° C to 40° C, the overall form of the spectrum changes drastically. There is an appreciable intensity reduction below -13.0 p.p.m. and the line at -11.6 p.p.m. has disappeared. After maintaining the sample for many hours at 40° C the resonances subsequently obtained at 26° C were broadened, presumably due to aggregation of the sample at this high concentration. So we could not make a direct intensity comparison between the 26° C and 40° C spectra. At 40° C the intensity in the low field part of the spectrum relative to the high field part is much lower than in the 26° C spectrum. On the basis of internally consistent intensities in the 40° C spectrum its integrated intensity corresponds to ~eight protons. Loss of about eight resonances is consistent, within experimental error, with the melting of either the *a* or the *b* region of

the structure proposed for the mRNA fragment (Fig. 1 of ref. 3). To interpret the results in more detail the spectra expected for the two proposed helices were calculated on the basis of the ring current shift contributions from nearest neighbours with the results given by the stick diagram at the bottom of Fig. 1.

The melting observed by NMR can be ascribed to a certain helical region only if at least one resonance can be assigned to the hydrogen-bonded NH proton of a particular base pair in that helical region. Since the accuracy of the calculation is no better than ~0.3 p.p.m.^{1,2} it is necessary to have a resonance which is separated by at least this distance from the rest of the spectrum before it can be unambiguously identified merely by comparing the calculated spectrum with the observed. Fortunately, such a line does appear near -11.6 p.p.m., corresponding to the GC43 (GC base pair involving nucleotide number 43 from the 5' end) and this is the only resonance calculated to be at fields higher than ~12.0 p.p.m. This assignment is independent of whether we assume that A44 is looped out so that GC45 stacks on GC43 giving a calculated position of -11.8 p.p.m. or that A44 is stacked between pairs 43 and 45 giving -11.4 p.p.m. for GC43. Although we cannot distinguish between these two possibilities now, the observed position of the GC43 resonance requires that one of these two conformations exists. The disappearance of the resonance at -11.6 p.p.m. in going from 26° C to 40° C suggests that resonances of the *b* helix are broadening in this temperature range. This conclusion is corroborated by the simultaneous intensity loss in the rest of the spectrum which coincides rather well with the reso-

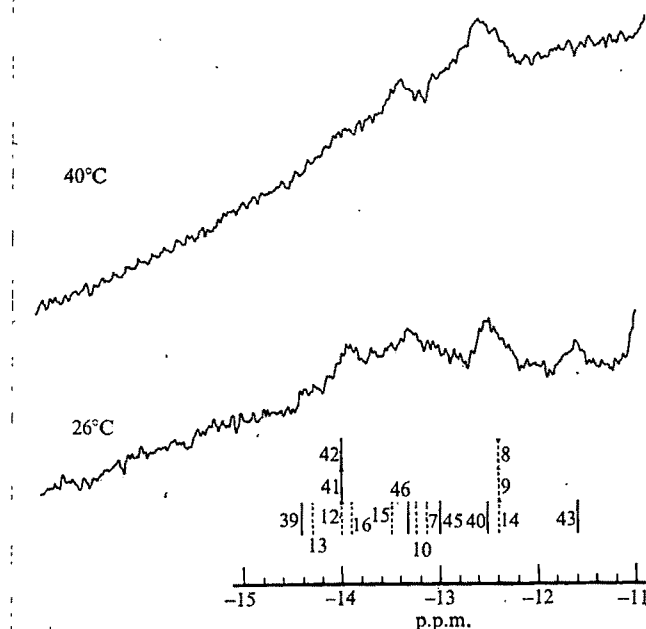


FIG. 1 The 300 MHz NMR spectra of the ring NH hydrogen-bonded protons of the R17 fragment at 26° C and 40° C. Positions are given in parts per million (p.p.m.) downfield from a DSS (2,2-dimethyl-2-silapentane-5-sulphonate) standard. The stick diagram at the bottom of the figure depicts the spectrum calculated for the secondary structure given in Fig. 1 of the previous paper³. The dashed lines correspond to the *a* helix which contains the termination codon for the coat protein gene, the solid lines to the *b* helix which contains the initiator codon for the replicase gene. Concentrations in the solution were: RNA fragment ~0.5 mM; NaCl, 50 mM; MgCl₂, 8 mM; ammonium cacodylate, 10 mM. The total sample volume was 0.025 ml in an approximately spherical NMR microcell. The R17 RNA fragment was prepared as described in Fig. 1, ref. 3. Examination of aliquots of the sample by polyacrylamide gel electrophoresis and by T₁ and pancreatic RNase fingerprinting showed it to be more than 95% pure.

nances expected from the other base pairs in the *b* helix. In particular, the loss of intensity observed around -13.1 p.p.m. is understandable if we include base pairs GC45 and GC46 in the *b* helix; without these two pairs no intensity decrease would be expected in this spectral region from the other five base pairs of helix *b*.

The NMR results on the R17 RNA fragment presented here support the interpretation of the melting behaviour given in the preceding paper³ in that the *b* helix is the first to melt, and in this way support the rules for thermodynamic stability developed to predict the secondary structure of RNAs⁷.

The results indicate that the AUG initiation triplet of the R17 replicase gene is buried in a base-paired region in this particular fragment. The combination of relaxation kinetics and NMR shows that while the fragment is predominantly base paired under physiological conditions, there is still a finite possibility of finding the helix open. Raising the temperature above 40°C would increase the fraction of time that helix *b* is open and in this way it might be possible to observe changes in the biological function of this fragment, that is initiation and translation, which could be correlated with changes in the helix-to-coil equilibrium of the helix containing the AUG initiator codon.

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Effect of camptothecin on simian virus 40 DNA

THE alkaloid camptothecin has been shown to be a potent, rapidly acting inhibitor of RNA and DNA synthesis in eukaryotic cells¹⁻⁵. The drug is selectively active against high molecular weight nuclear RNA synthesis²⁻⁵, having no effect on mitochondrial⁶ or 4-5S RNA synthesis^{2,5}. Its effect on RNA synthesis is rapidly reversible^{1-3,5}. In addition, it has been shown that camptothecin induces alkali-labile links in DNA^{1,7-9} which are rapidly reversed by removal of the drug⁹. It was suggested that this action on DNA is responsible for the effect of the drug on RNA synthesis⁵.

Here we describe the effect of camptothecin on simian virus 40 (SV40) DNA. These molecules normally exist as either covalently closed superhelical double-stranded circles (component I) or relaxed circles containing single-strand scissions (component II). Introduction of even one single-strand break in component I molecules can be easily assayed because of the drastic change in sedimentation coefficient

(from 53S for component I to 16-18S for component II) seen during centrifugation in alkaline gradients. Such analysis shows the major effect of camptothecin treatment to be the introduction of one single-strand break or alkali-labile region per component I molecule affected.

To study the effect of camptothecin on SV40 DNA, labelled viral DNA from infected drug-treated cells was analysed by

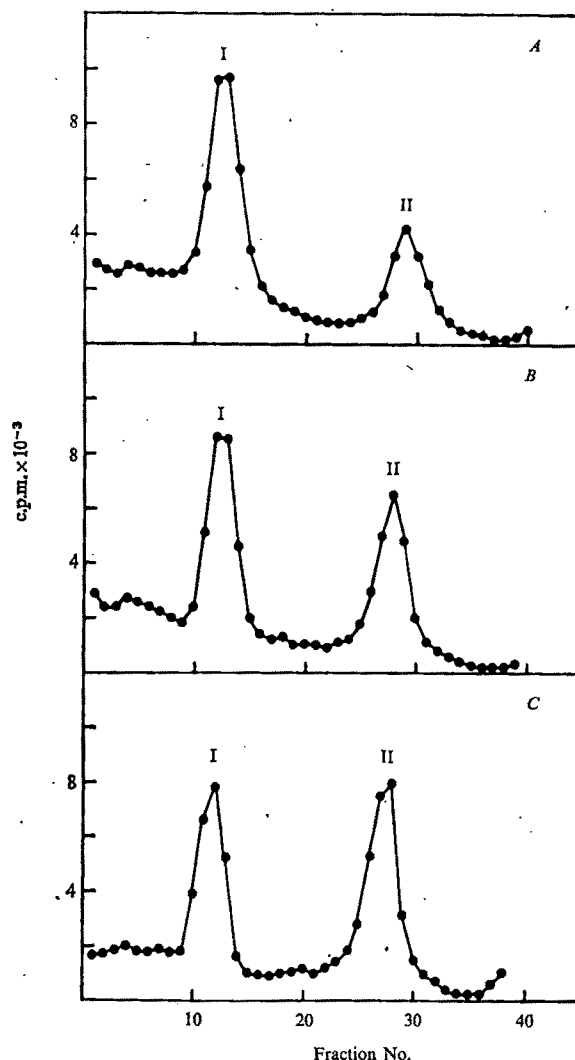


Fig. 1 Velocity sedimentation in alkaline CsCl of viral DNA extracted from cells treated with camptothecin. Confluent monolayers of Vero cells, grown in 60 mm Petri plates in Dulbecco-Vogt medium with 10% Colorado calf serum, were infected with SV40 (small plaque strain) at a multiplicity of approximately 2 plaque-forming units (PFU) per cell. The virus was allowed to adsorb to the cells for 3 h at 37°C , at which time the inoculum was replaced with medium and the incubation continued at 37°C . Fresh medium containing $20\ \mu\text{Ci ml}^{-1}$ ^3H -thymidine (Mallinckrodt, $14.6\ \text{Ci mM}^{-1}$) was added at 45 h after infection, and the incubation was continued 3 h, at which time viral DNA from one plate (A) was immediately extracted by the method of Hirt¹². Other plates were treated for 30 min at 37°C with $10\ \mu\text{g ml}^{-1}$ camptothecin (B) or $100\ \mu\text{g ml}^{-1}$ camptothecin (C) in fresh medium, followed by Hirt extraction using lysing buffer containing $5\ \mu\text{g ml}^{-1}$ camptothecin. Aliquots (0.1 ml) of the Hirt supernatants were layered on 3 ml CsCl ($\rho = 1.52$) containing $0.1\ \text{M K}_2\text{HPO}_4$ (pH 12.9), and centrifuged at 15°C for 80 min in an SW 50.1 rotor at 44,000 r.p.m. The tubes were punctured and fractions were collected as described¹⁴ on 22.8 cm diameter, segmented Whatman GF/A filter discs which were dried, then washed in a Büchner funnel with 6% trichloroacetic acid and absolute ethanol. Segments containing individual fractions were then pulled off with forceps, dried in scintillation vials, and counted in toluene-Liquiflour scintillation fluid. Sedimentation is from right to left.

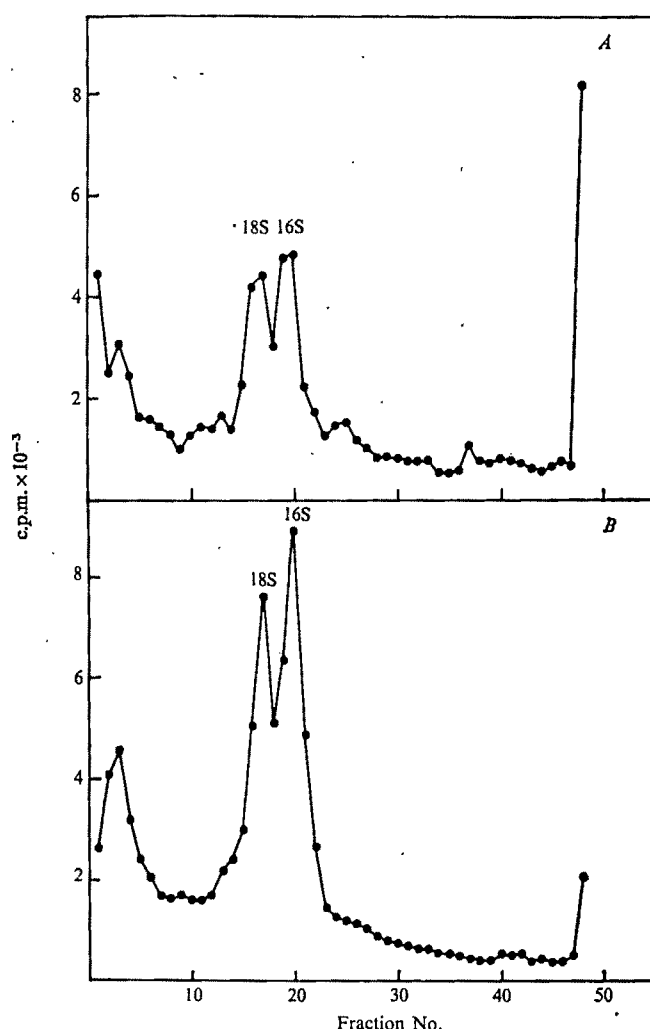


FIG. 2 Velocity sedimentation in alkaline sucrose of viral DNA extracted from camptothecin-treated cells. Aliquots (0.2 ml) of Hirt supernatants of the untreated cells (A) or the cells treated for 30 min with 100 µg ml⁻¹ camptothecin (B) (see legend to Fig. 1) were layered on 11 ml 10–30% sucrose gradients containing 0.3 M NaOH, 0.7 M NaCl, 0.001 M EDTA. Centrifugation was for 16.5 h at 10° C in an SW41 rotor at 40,000 r.p.m. Fractions were collected on GF/B filters (see legend to Fig. 1). Sedimentation is from right to left.

alkaline caesium chloride centrifugation. As shown in Fig. 1A, viral DNA sediments as two bands in such gradients, component I at 53S and component II at 16–18S. Component I comprises the majority of the viral DNA molecules in normal conditions, with component II arising mainly from the random introduction of one single-strand break per component I molecule¹⁰.

When infected cells making viral DNA are treated with camptothecin for a short period of time, some of the component I molecules are converted to component II. Treatment of cells containing labelled viral DNA for 30 min with 10 µg ml⁻¹ camptothecin (Fig. 1B) produces some conversion, an effect which can be heightened by increasing the concentration of camptothecin to 100 µg ml⁻¹ (Figure 1C). As shown in Table 1, up to one-third of the component I molecules are converted to component II in these conditions. (Incubation without camptothecin causes no appreciable change in the distribution of components I and II.)

To characterise the mode of action of camptothecin further, viral DNA from treated and control cultures was centrifuged through alkaline sucrose gradients in conditions which allow the partial resolution of the separated circular (18S) and linear (16S) strands of component II (Fig. 2). Consistent

TABLE 1 Component I molecules converted to component II

Camptothecin treatment	% Component I	% Component II	% Component I converted to component II
None	67	33	—
10 µg ml ⁻¹ , 30 min	54	46	19
100 µg ml ⁻¹ , 30 min	44	56	34

Areas in Fig. 1 were determined under component I and component II peaks by the method of Girard, *et al.*¹². Values are expressed as % of total component I plus component II.

with the results in Fig. 1, there is an increase in radioactivity in both the 18S and 16S peaks in the camptothecin-treated sample. (The amount of component I cannot be estimated as in these conditions some of the molecules pellet.) Quantita-

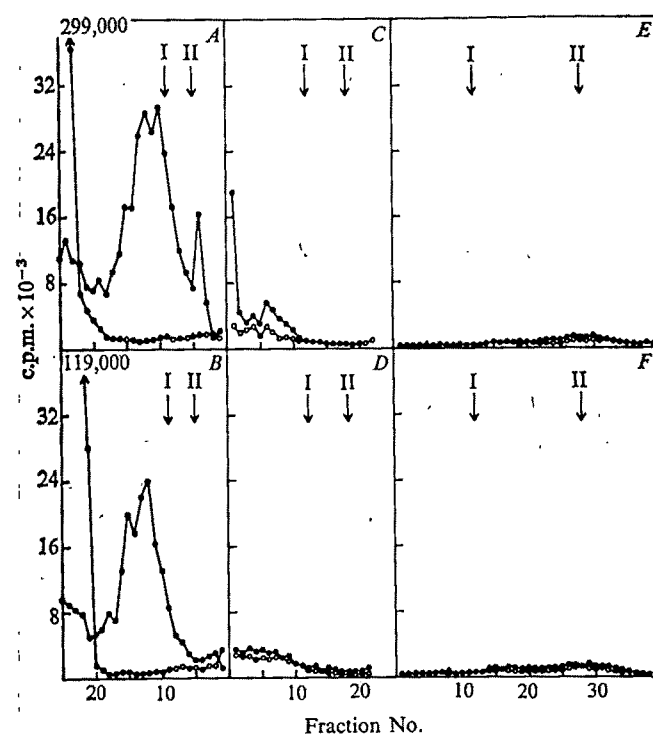


FIG. 3 Velocity sedimentation in alkaline gradients of cellular DNA from camptothecin-treated cells. Vero cells were labelled with 10 µCi ml⁻¹ ³H-thymidine for the 12 h before infection. At 48 h post-infection, cultures were treated with 100 µg ml⁻¹ camptothecin for 30 min, removed from the plates with trypsin-EDTA, and layered on alkaline sucrose gradients (A and B), as described by Abelson and Penman⁹. The 10 ml gradients containing 15–30% sucrose in 0.2 M NaOH, 1 M NaCl, 0.01 M EDTA, were prepared on top of a 0.5 ml cushion of 35% CsCl in the same buffer. Then 0.5 ml of lysing solution (1% Sarkosyl; 0.2 M NaOH, 5% sucrose) was layered on top of the gradient, followed by the suspension of whole cells. Parallel cultures were extracted by the method of Hirt¹². Aliquots (0.2 ml) of the Hirt supernatants were layered on the same type of gradients (C and D) or on alkaline CsCl (E and F). The alkaline sucrose gradients were centrifuged for 2 h at 40,000 r.p.m. in an SW 41 rotor at 24° C. Centrifugation of the alkaline CsCl was for 80 min at 44,000 r.p.m. in an SW 50.1 rotor at 15° C. The positions of the SV40 component I and II DNA added as external markers are indicated. A, C and E, DNA from infected cells; B, D and F, DNA from mock-infected cells. ●, camptothecin-treated cells; ○, untreated cells. Fractions were collected from the top using the Buchler Auto Densi-Flow (A and B), or from the bottom by puncturing the tube (C–F), then assayed on GF/B (A–D) or GF/A (E and F) filters (see legend to Fig. 1). In gradients displaying the centrifugation of aliquots of Hirt supernatants (C–F), the axes were corrected to represent the radioactivity present in the entire Hirt supernatant. Sedimentation is from right to left in all panels.

tion of the approximate areas under the 16S and 18S peaks demonstrates that they are in the same relative proportions in the camptothecin-treated sample as in the untreated control. This would not have resulted had the affected molecules received either a double-strand break or two single-strand breaks in opposite strands. In either case a preponderance of 16S linear molecules would have been observed.

Furthermore, there are no new lower molecular weight discrete species discernable due to drug treatment, as would be expected if the drug caused two or more breaks at specific sites on a single strand. It is possible, however, that the single-strand breaks caused by the drug are randomly located on the viral DNA. If this were the case, the result of multiple breaks would be relatively small increases in the ratio of 16S to 18S DNA, and in the heterodisperse, lower molecular weight background. We would not detect these changes in our experiments.

It might be argued that cellular DNA, which is broken or rendered alkali-labile by camptothecin treatment^{1,7-9}, would appear in the extracts, complicating our results. The patterns shown in Figs 1 and 2 give no indication that pulse-labelled cellular DNA, synthesised after infection, is extracted and sediments near viral DNA in alkaline conditions. We have also tested the effect of the drug on cellular DNA synthesised before the infection. The results presented in Fig. 3 show that pre-labelled cellular DNA is broken (or rendered alkali-labile) by the drug, and sediments in alkali only slightly faster than viral DNA (Fig. 3, A and B), but is removed or repaired during the extraction (Fig. 3, C-F).

We have shown that the principal change in SV40 DNA caused by camptothecin is the production of one single-strand break or alkali-labile region in the molecule. It is possible that additional breaks may occur at random locations in the molecule. These experiments with closed circular DNA constitute the first direct indication that the drug affects only one DNA strand, rather than inducing double-stranded alkali-labile regions.

It has still to be determined if the specificity of the camptothecin effect on SV40 DNA is due to the drug's preference for a particular sequence of nucleotides, similar to the mode of action of restriction endonucleases¹¹, or if the drug can cause a scission at any site on the molecule. In addition, it is still unclear whether the drug's effect on SV40 DNA is the induction of single-strand breaks or alkali-labile links, as has been suggested for cellular DNA by Abelson and Penman⁹. Further experiments are under way to determine the mechanism of action of camptothecin on SV40 DNA.

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Localisation of Monocyte Binding Site of Human Immunoglobulin G

THE binding of IgG to macrophages is thought to be important in both the afferent and efferent limbs of the immune response¹⁻³. Berken and Benacerraf⁴ studied the properties of guinea pig IgG2 antibodies cytophilic for macrophages and concluded that the binding site was in the Fc region. Similarly Lo Buglio *et al.*⁵ and Abramson *et al.*⁶ inhibited with isolated Fc fragment the binding of human monocytes to red cells coated with anti-D. Abramson *et al.*⁶ also studied the inhibitory activity of various fragments obtained by peptic digestion of IgG. The F(ab')₂ fragment was slightly inhibitory, unlike Fab fragment, and the authors suggested that the binding site was in the N-terminal portion of the Fc fragment (now known as the C_H2 region). Huber and Fudenberg⁷ reached similar conclusions following experiments in which F(ab')₂ fragments of anti-D antibodies were attached to red blood cells and produced rosettes with human monocytes, although the possibility of contaminating IgG could not be eliminated. In contrast, MacLennan *et al.*⁸ were unable to inhibit neutrophil phagocytosis of sensitised bacteria with immune complexes of antigen and the F_{ab} fragment of rabbit IgG. Since the F_{ab} fragment includes the Fab and C_H2 regions but lacks the C_H3 region this suggests that the latter may be the site of neutrophil binding. Furthermore, Yasmien *et al.*⁹, working with a

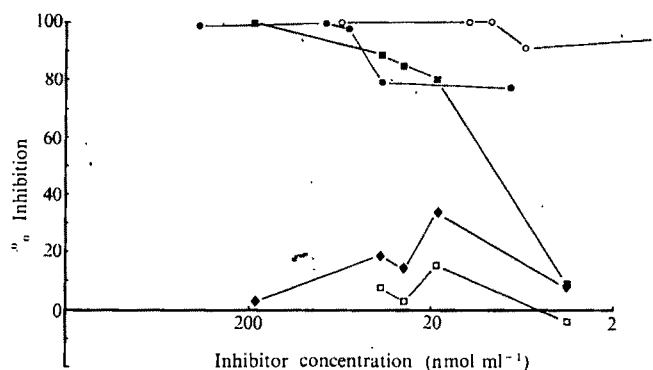


Fig. 1 Percentage inhibition of monocyte rosette formation obtained with different molar concentrations of IgG, various subfragments of IgG and human serum albumin. % Inhibition was obtained from the formula:

$$100 - \left[\frac{\frac{\text{Number of rosetting cells in test}}{\text{Number of monocytes in test}}}{\frac{\text{Number of rosetting cells in control}}{\text{Number of monocytes in control}}} \times 100 \right]$$

where the control consisted of cells and medium only. The curves represent values obtained in a single experiment. Similar values were obtained in a duplicate experiment. ○, Intact IgG; ●, pFc' fragment; ■, Fc fragment; ♦, Fab fragment; □, human serum albumin.

heterologous system (human immunoglobulin G and guinea pig macrophages) have presented evidence that macrophage binding is a property of the C_H3 region of the molecule. In view of these conflicting reports we have investigated the possible role of the C_H3 region of human IgG in binding to homologous monocytes.

Human monocytes were prepared essentially as described by Hay *et al.*¹⁰ and resuspended to a final concentration of 4×10^5 cells ml⁻¹ in medium lacking foetal calf serum (FCS). The cell suspension (0.5 ml = 2×10^5 monocytes) was distributed in equal quantities into chambers consisting of lucite rings affixed to 22 mm square glass coverslips. The chambers were placed in a humidified airtight box fitted with inlet and outlet tubes. The latter were used to introduce an 8% CO₂-air mixture into the box. After incubation for 1 h at 37° C each chamber was washed once with medium lacking FCS to remove cells not adhering to the walls of the chamber.

For the inhibition experiments Fc and Fab fragments of pooled human IgG (obtained from Kabi A. B., Stockholm) were prepared by papain digestion¹¹ and isolated by ion-exchange chromatography¹². The pFc' fragment of pooled IgG (i.e., the C_H3 region) was prepared by pepsin digestion and isolated by Sephadex G-150 gel filtration¹³. Similar series of different concentrations of each fragment were prepared (assuming E₁^{1%}_{cm} 280 nm. of 14.6), and 0.5 ml of each preparation was added to a chamber. Every sample was duplicated and a control consisting of 0.5 ml of medium (without FCS) was also included. The chambers were flushed with 8% CO₂-air and incubated at 37° C for 1 h. The chambers were washed twice with medium and 0.5 ml of a 0.5% suspension of human red cells, previously coated with incomplete anti-D, was added to each. After flushing again with 8% CO₂-air the chambers were incubated at 37° C for 30 min and finally at 4° C for a further 30 min. Each chamber was then filled with medium, covered with a microscope slide and inverted to bring the coverslip uppermost. The degree of rosette formation was then assessed by phase contrast microscopy. Approximately 100 cells were counted in each chamber and monocytes binding two or more red cells were counted as rosettes.

Figure 1 shows the percentage inhibition of monocyte rosette formation obtained in a single experiment with different concentrations of pooled human IgG, Fc, Fab and pFc' subfragments of IgG and human serum albumin. All inhibitors were studied over the concentration range 0.02–1.0 mg ml⁻¹, but since the Fc and Fab fragments represent only one third, and the pFc' fragment one sixth, of the intact IgG molecule, the data in Fig. 1 are expressed on a molar basis. At concentrations between 20 and 200 nmol ml⁻¹ of each inhibitor the pFc' fragment, the Fc fragment and whole IgG showed levels of inhibition between 80% and 100% whereas HSA and Fab fragments showed values of less than 20%. Similar results were obtained over the same concentration range when the same protein preparations were tested in a second study. The percentage inhibition obtained with the different proteins and fragments (used at a concentration of 1 mg ml⁻¹, which discriminated readily between Fc and

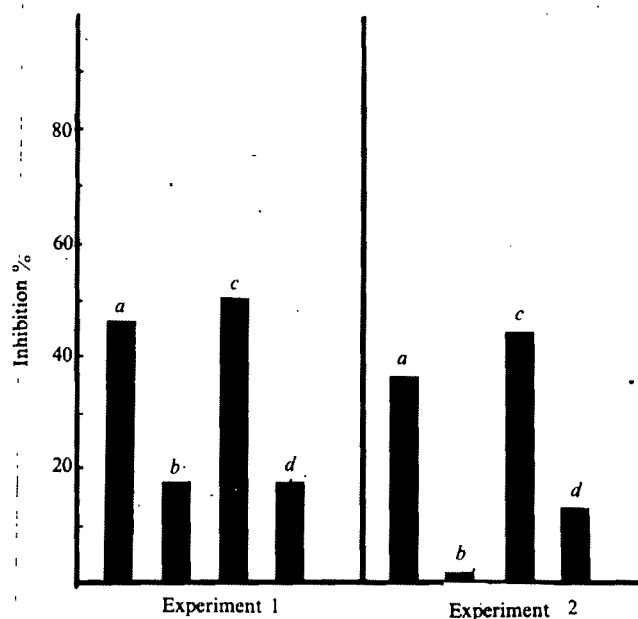


Fig. 2 Percentage inhibition of monocyte rosette formation obtained with preparations of pFc' fragment from, a, human IgG1; b, IgG2; c, IgG3; and, d, IgG4 myeloma proteins. Inhibitor proteins were prepared as previously described¹⁴ and detailed chemical characterisation is presented elsewhere¹⁵. The results of two separate experiments are plotted.

Fab fragments) in these and other replicate experiments are shown in Table 1. These results suggest that the binding site for homologous macrophage fixation is located in the pFc' region of the IgG molecule, as was also recently reported for heterologous interactions⁹.

In additional experiments inhibition of rosette formation was investigated with pFc' fragments isolated from all four subclasses of human IgG. Two IgG1 myeloma proteins and single samples of IgG2, IgG3 and IgG4 myeloma proteins were digested with pepsin to yield pFc' fragments and the fragments isolated by Sephadex G-150 gel filtration¹⁴. The results of two separate inhibition experiments are shown in Fig. 2. The pFc' fragments from IgG1 and IgG3 myeloma proteins inhibited rosette formation more strongly than did

Table 1 Inhibition of Monocyte Rosette Formation by Pooled Human IgG and Various Subfragments

Inhibitor*	% Inhibition obtained in different experiments†		
IgG	100	100	100
Fc fragment	100	100	
Fab fragment	20	9	8
pFc' fragment	100	99	
Human serum albumin	30	2	

* All preparations used at 1 mg ml⁻¹ concentration.

† Calculated as in Fig. 1, and includes data from Fig. 1.

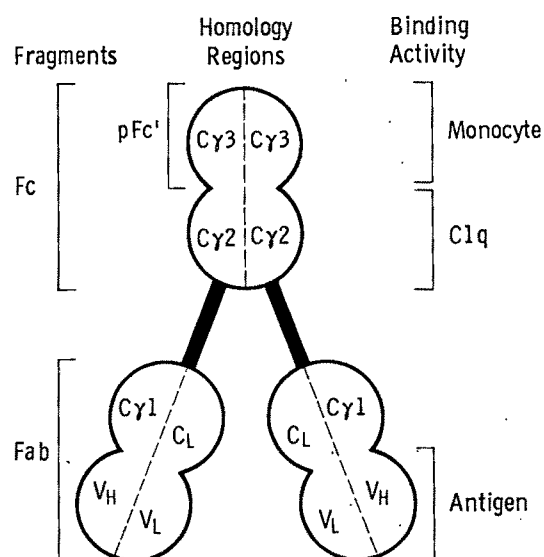


Fig. 3 Schematic model of human IgG molecule showing the binding activities associated with the different homology regions.

pFc' fragments from IgG2 and IgG4 myeloma proteins. The subclass distribution observed with pFc' fragments parallels exactly the results obtained using subclass proteins^{6,10,16}. None of the preparations, however, inhibited as well as pFc' fragment from pooled IgG. A possible explanation of this observation is that there are different binding sites for the different IgG subclasses, either on the same cell or on different cells. But such an explanation conflicts with the conclusions of Huber *et al.*¹⁶, who studied the monocyte binding activity of intact subclass proteins.

The possibility that the monocyte receptor interacts with aggregated rather than native pFc' fragment was investigated in the following manner. A concentrated preparation of pFc' fragment was chromatographed on Sephadex G-150 and a fraction of the eluted peak ($S_{20,w}=2.3$) was taken directly, without any concentrating procedure, for ultracentrifugal analysis in the MSE Centriscan at 60,000 r.p.m. Monocyte rosette inhibition was assayed simultaneously on the same sample. This material, which was shown to be fully inhibitory, contained no detectable aggregates (threshold of detectability 1.5% of total protein). Furthermore radio-labelled monomeric pFc' fragment was concentrated by three different procedures (ultrafiltration, lyophilisation and polyethylene glycol) and rechromatographed on Sephadex G-150. Aggregated material, if present, represented less than 0.25% of the total counts applied to the column. We therefore conclude that the monocyte receptor probably has specificity for native IgG rather than denatured or aggregated IgG.

Our study is further evidence for Edelman's 'domain concept'^{17,18} that the homology regions of the individual peptide chains of IgG interact to form six globular units each of which has evolved to perform a distinct function. Binding sites for antigen, for the C1q component of complement and for monocytes have now been localised to separate homology regions (see Fig. 3), and the importance of each of these activities might be expected to exert strong evolutionary pressure for the conservation of each binding site.

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Antibody response and virus survival in cats vaccinated against feline leukaemia

We have shown that leukaemia virus can be transmitted from cat to cat by contact or aerosol¹. There is also substantial epizootiological evidence that such transmission occurs in natural conditions^{2,3}. Horizontal transmission occurs because feline leukaemia virus (FeLV) replicates not only in cells of the haematopoietic system but is also found in large amounts in respiratory and alimentary mucous membranes and is excreted in the urine⁴. It is therefore not surprising that the infection in cats is fairly common. In the City of Glasgow we found that about one third of the cats sampled had antibodies against FeLV (mean titre = 2; ref. 4). We recently examined 100 young cats from the surrounding countryside and found antibody in six (mean titre = 20). These facts indicate that vaccination would be valuable and we have evidence that it is feasible. Here we report on the use of FeLV-infected cells to produce an immune response of a high level in cats.

The antibodies being measured are directed against the virus subgroup specific glycoprotein antigens which are located on the membrane of the infected cell and on the envelope of the virus; they have the same specificity as those responsible for virus neutralisation and cell cytotoxicity (W. J., L. M., O. J., H. L., and C. H., unpublished). An indirect immunofluorescence test is used in which the target cells are living, virus-infected, feline lymphoblasts which are cultured in suspension^{4,5}.

We inoculated cats with infected cells for two main reasons. First, in the feline sarcoma virus (FeSV) system, high antibody titres have been associated with either failure of tumour induction or regression of established tumours⁶. Similar results have not been reported in feline leukaemia. We therefore tried to mimic the sarcoma situation by injecting cats with packs of fibroblasts which were actively replicating FeLV from their cell membranes. This was on the assumption that to obtain high antibody levels it might be necessary to present cell walls bearing antigen arrays to

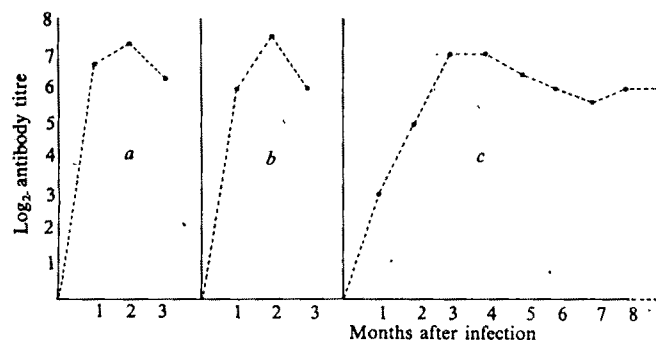


Fig. 1 Group mean antibody responses in three groups of cats inoculated with FeLV-infected cells. Group a received 4×10^7 feline embryo (FEA) monolayer cells; group b were given 4×10^7 feline lymphoblastoid cells grown in suspension, and group c received 2×10^9 FEA cells.

TABLE 1 Type and number of cells infected with various subgroups of FeLV used to immunise cats, the peak antibody titres induced the relationship of the immune response to the establishment or persistence of virus infection

Cat	Type	Cells No.	Peak antibody titre	Duration of experiment (months)	Virus isolation after necropsy	Virus subgroups
1	FEA*	2×10^9	256	13	+	A + B
2	FEA	2×10^9	128	13	+	A + B
3	FEA†	2×10^9	32	6	+	A + B
4	FEA†	2×10^9	64	6	+	A + B
5	FEA§	2×10^9	256	6	+	A
6	FEA§	7×10^8	128	6	+	A
7	FEA§	7×10^8	64	6	+	A
8	FEA	4×10^7	128	1	0	A + B + C
9	FEA	4×10^7	32	1	0	A + B + C
10	FEA	4×10^7	16	1	0	A + B + C
11	FEA	4×10^7	128	1	0	A + B + C
12	FEA	4×10^7	256	3	0	A + B + C
13	FEA	4×10^7	32	3	0	A + B + C
14	FEA	4×10^7	512	3	0	A + B + C
15	FEA	4×10^7	512	3	0	A + B + C
16	FL†	4×10^7	16	1	0	A + B + C
17	FL	4×10^7	128	1	0	A + B + C
18	FL	4×10^7	512	1	0	A + B + C
19	FL	4×10^7	64	3	0	A + B + C
20	FL	4×10^7	256	3	0	A + B + C
21	FL	4×10^7	512	3	0	A + B + C

Cats were approximately 6 months old at the start of the experiment and had no demonstrable specific antibody at that time.

* FEA, feline embryo fibroblasts grown as monolayers.

† FL, feline lymphoblasts grown in suspension.

‡ Simultaneous administration of 4×10^{10} *B. pertussis*.

§ Simultaneous administration of 4×10^{10} *B. pertussis* and 1 ml Freund's complete adjuvant.

the immune system. Second, we considered it likely that the integrity of the glycoprotein complexes which are the effective immunogens might be well preserved by this presentation.

Feline embryonic fibroblast cells of the FEA strain were grown as monolayers (FE) and were infected with FeLV as previously described⁷; isolates containing various FeLV subgroups (A; A + B; and A + B + C) were used⁸. Cells were collected, washed three times in Alsever's solution and pelleted by low speed centrifugation. Minimal buffer was added to enable the cell pack to pass through an 18 gauge needle for subcutaneous injection.

Serum samples were taken before inoculation and afterwards at monthly intervals. Detailed haematological examinations were made to check for the possible development of anaemia, leukaemia or the thymic deficiency and immunosuppression syndrome, all of which are caused by FeLV infection. When the animals were killed they were examined for the presence of FeLV by two methods. Preparations of bone marrow and tracheal epithelium, the two most sensitive monitoring sites², were examined electron microscopically (EM). Bone marrow cells were also co-cultivated with FE cells growing as monolayers. The latter were then subcultured every 3 to 4 d for 21 d. The cells were divided; one aliquot was used to prepare sections for EM examination and the other was examined for the presence of FeLV group specific antigen by an indirect immunofluorescence test using goat anti-FeLV antiserum¹.

The results are shown in Table 1. When 2×10^9 cells were given, high antibody titres resulted and these persisted for at least 13 months. It will be seen from Fig. 1c that these cats did not reach their maximum antibody titre for 3 months; after they were killed, virus was found in the bone marrow. In contrast, cats which were inoculated with 4×10^7 cells (cats 8 to 15), produced good antibody response by 1 month after inoculation (Fig. 1a) and no virus was found in tissues taken at necropsy. At no time did any of these animals show a clinical or haematological abnormality

and no lesions were seen at necropsy or found on histological examination.

This experiment was repeated (Table 1, cats 16 to 21) using infected feline lymphoblasts grown in suspension culture; again a mean antibody titre of 128 was obtained. Virus was not detected at autopsy 1 or 3 months after inoculation and the cats remained healthy throughout. It is obvious from Fig. 1a and b that a rapid antibody response occurred in the first month to near peak titre. It is not known if these cats became infected during the first month or if the infection was suppressed by the rapidly rising antibody level. We are currently carrying out a larger experiment to allow weekly monitoring for infection during the first month. It would appear, however, that it is possible to obtain high titres using infected cells. In the small number of cases in which adjuvants were incorporated (Table 1) there was no apparent benefit and the peak antibody titre was not reached until the 4th month.

We have found that if cats which have naturally acquired specific antibody are inoculated with infected cells or purified virus, a secondary response results. The animal in Fig. 2a, which was typical of several, had a naturally occurring titre of 16. It was inoculated with 4×10^7 FeLV-infected FE cells and showed a rapid and high response. Virus could not be detected after autopsy. Another case from a different experiment is shown in Fig. 2b. This cat had a preinoculation titre of 8. It was given intravenously a purified FeLV preparation containing approximately 10^{10} virus particles when it was 4 months of age. There was viraemia between months 3 and 7, but during this period there was an antibody rise. The animal remained healthy until killed; there was no demonstrable virus in samples taken at autopsy. At the start of this experiment, none of the other nine animals involved had antibody. All became infected and three developed leukaemia during the limited time span of the experiment. The low antibody response of this group is shown in Fig. 2b.

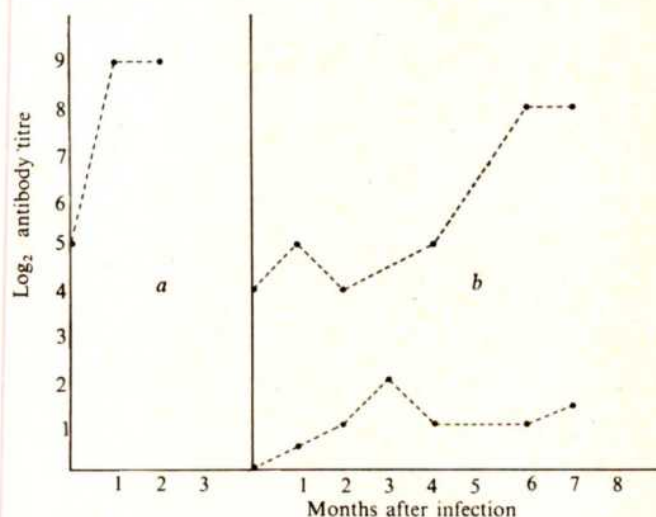


Fig. 2 a, This young adult cat had 5 antibody units acquired by natural exposure to infection. It was inoculated with 4×10^7 FeLV-infected FEA cells subcutaneously. A high antibody titre was found at 1 month. No virus could be detected when the animal was killed after 2 months. b, A young adult cat had 4 naturally acquired antibody units. It was infected with a high dose of gradient purified virus which is normally 100% pathogenic. Viraemia was present after 3 months. Virus then disappeared and none was found at autopsy. At this time a high antibody level was present. c, Gives the mean antibody response of the other nine cats in this experiment. None had antibody titres before inoculation with virus and all became infected; three developed leukaemia before the experiment was terminated.

The following conclusions may be drawn from these results. If the cat had previous experience of FeLV and had responded immunologically even to a mild degree, a secondary response occurred and no persistent infection resulted. If the virus input dose was high, the animal became infected but subsequently suppressed the infection. Cats with no previous experience of infection seemed to have a cell dose-related response. The higher doses induced a good antibody level but this did not terminate the infection, even when observed over a long period. The persistence of infection may have been associated with the slower rate of antibody rise and its inability to prevent the establishment of infection of epithelial cells of many tissues¹. These cells may not be susceptible to the action of cytotoxic antibody. In the groups given lower doses the antibody rise took place in the first month and virus was not found in the animals at that time or later.

The mean antibody levels achieved in these experiments are considerably higher than those seen in cats with naturally acquired infections. The highest titre we have seen in a spontaneous feline leukaemia case was 32, and this was exceptional. Seventy-five per cent of cases had no demonstrable antibodies and the mean level of those which had was 8. In our surveys of normal cats, the highest titre seen in those which had antibodies was 64 and the mean was 4.

These results indicate the feasibility of vaccination against leukaemia by inducing either large primary responses or small primary and large secondary responses. As leukaemia and its associated diseases are not usually found in immature cats and as most animals seem to become infected horizontally by contact later in life^{1,2}, the time scale of the natural history of the disease is suitable for the application of immunisation. We have already obtained significant antibody levels with inactivated vaccines; these experiments will be reported later.

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Two Distinct Types of Capping of Surface Receptors on Mouse Lymphoid Cells

IMMUNOGLOBULIN (Ig) molecules on the surfaces of metabolically active mouse lymphocytes are redistributed into "caps" when crosslinked with fluorescent, ferritin-conjugated or ¹²⁵I-labelled anti-Ig antibody at room temperature or 37°C¹⁻³. Caps of labelled Ig-anti-Ig complexes form over the Golgi region of the cell, and are rapidly ingested by endocytosis^{2,3}. Capping of receptors for the plant lectin concanavalin A

(con A) on mouse lymphoid cells is also accompanied by considerable endocytosis of label^{3,4}. On the other hand, little or no internalisation of label occurs after capping of H-2^{1,3} and Thy-1 (θ)¹ alloantigens on mouse lymphocytes and thymocytes, and anti-lymphocyte globulin receptors on rat spleen cells³.

To better define the relationship between cap formation and endocytosis, we examined capping of surface Ig, con A receptors, and H-2, Thy-1 and TL alloantigens on mouse thymus and spleen cells by immuno-electron microscopy and immunofluorescence. We report that caps form primarily in two distinct locations on the cell surface, either over or directly opposite the Golgi region. Location of capping seems to be determined more by cell type than by surface receptor, with caps generally forming over the Golgi region of spleen cells, and opposite the Golgi region of thymus cells. Caps over the Golgi region are extensively internalised by endocytosis, but there is little or no internalisation of caps forming opposite the Golgi region. Furthermore, cap formation over the Golgi region was partially inhibited by cytochalasin B, while capping opposite the Golgi was unaffected.

For these studies, mice of the C57Bl/6/TL+ congenic stock were used. Suspensions of thymus and spleen cells with greater than 90% viability (determined by trypan blue exclusion) were prepared in medium 199 supplemented with 5% (v/v) gamma globulin-free foetal bovine serum, and cells were incubated and washed in this medium. All incubations were for 30 min at 37°C, with cells maintained in suspension by periodic agitation. Cells were washed twice by centrifugation at 4°C after each incubation.

The following mouse alloantisera were used to label H-2, Thy-1 and TL alloantigens: (C57Bl/6/H-2^k × A)F₁ anti-C57Bl ascites leukaemia EL4 (anti-H-2^b), (A/Thy-1.1 × AKR/H-2^b)F₁ anti-A strain spontaneous leukaemia ASL1 (anti-Thy-1.2), and (A/TL- × C57Bl/6)F₁ anti-ASL1 (anti-TL.1,2,3).

Alloantigens on thymus cells were labelled for immuno-electron microscopy by incubating cells sequentially with the

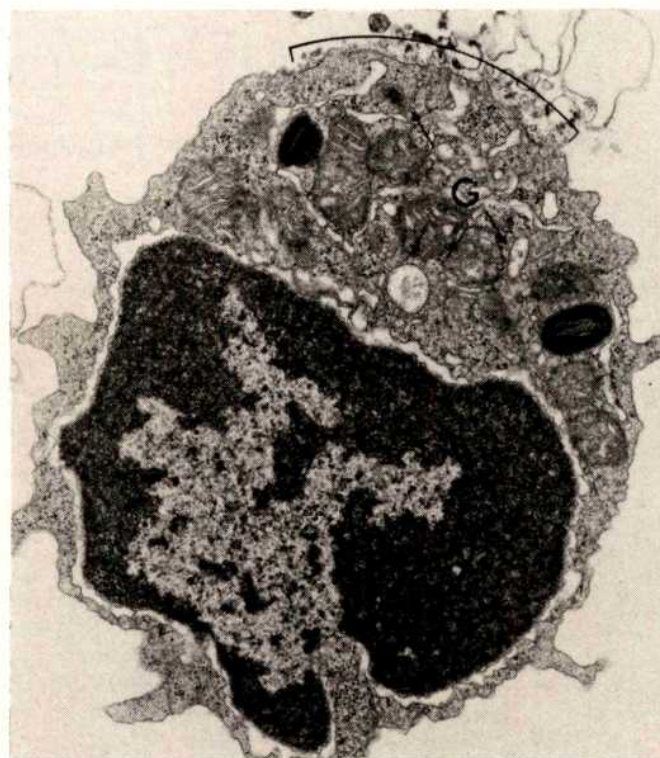


Fig. 1 Section through the approximate centre of a spleen cell, with a cap of con A receptors, labelled with con A, hybrid anti-con A/anti-ferritin antibody, and ferritin, directly over the Golgi region (G). The cap of ferritin is marked by a bracket, and label internalised in vesicles is indicated by arrows. (×17,100.)

Table 1 Site of Cap Formation and Amount of Endocytosis of Cell Surface Receptors

Receptor	Cell	Over Golgi	No. of caps		Endocytosis of label
			Opposite Golgi	Intermediate	
Surface immunoglobulin	Spleen	28	0	2	++++
Con A receptors	Spleen	10	2	0	++++
H-2 alloantigen	Thymus	3	12	5	±
	Spleen	10	11	8	++
Thy-1 alloantigen	Thymus	1	9	2	±
	Spleen	12	2	0	+++
TL alloantigen	Thymus	2	18	5	—

appropriate alloantiserum (anti-H-2 and anti-Thy-1 were diluted 1:10, and anti-TL 1:100), then with the hybrid rabbit F(ab')₂ antibody, anti-mouse IgG/anti-ferritin⁴, and finally with ferritin. Hybrid antibody induces cap formation in the same manner, and nearly as effectively, as bivalent antibody⁵. Hybrid antibody directed against the Fc fragment of mouse IgG (anti-mouse Fc/anti-ferritin) was used on spleen cells, to discern applied alloantibody from naturally-occurring surface Ig⁶. Surface Ig was labelled with hybrid anti-mouse IgG/anti-ferritin and ferritin, and con A receptors with purified con A⁷ (100 µg ml⁻¹), hybrid anti-con A/anti-ferritin antibody, and ferritin. Hybrid antibodies and ferritin were used at a concentration of 100 µg ml⁻¹.

For immunofluorescence, alloantigens were labelled with alloantiserum and either 300 µg ml⁻¹ of fluorescent rabbit anti-mouse IgG (on thymus cells) or 250 µg ml⁻¹ of fluorescent rabbit anti-Fc fragment of mouse IgG (on spleen cells). Surface Ig was labelled with fluorescent rabbit anti-mouse IgG (130 µg ml⁻¹), and con A receptors with con A and fluorescent rabbit anti-con A (200 µg ml⁻¹). The purified IgG fraction of rabbit antiserum was coupled to fluorescein isothiocyanate by the method of Cebra and Goldstein⁸.

To determine the site of cap formation relative to the Golgi region of the cell, and to estimate the amount of internalisation of label, cell suspensions were first labelled for immuno-electron microscopy and then allowed to settle onto a carbon-coated glass surface before preparation for electron microscopy. We found previously⁹ that lymphoid cells usually settle onto a surface with the Golgi region oriented to one side. Serial sections were cut parallel to the plane of the monolayer, from which sections through the approximate centre of the cell, including the Golgi region, were selected for analysis. The approximate amount of endocytosis of label was estimated from the number of cytoplasmic vesicles containing ferritin. Results are presented in Table 1.

Surface Ig and con A receptors capped over the Golgi region on 93% and 83%, respectively, of spleen cells analysed, and the caps were extensively internalised (Fig. 1). In contrast, H-2, Thy-1 and TL alloantigens capped over the Golgi region on only 15%, 9% and 8% of thymus cells, respectively. Caps on these cells formed predominantly directly opposite the Golgi region, and very little endocytosis of label was observed (Fig. 2). Although Thy-1 alloantigen capped primarily opposite the Golgi region on thymus cells, 86% of caps of the same alloantigen on spleen cells formed over the Golgi region, and there was considerable endocytosis of label. These results suggest that the cell type, rather than the receptor, determines the cellular site of cap formation, and that the amount of endocytosis depends on the site of cap formation. Capping of H-2 alloantigen on spleen cells was not polarised, and endocytosis occurred to a moderate extent, primarily in cells with caps in the vicinity of the Golgi region. Capping of con A receptors on thymus cells occurs too infrequently for accurate determination of the predominant site of cap formation by immuno-electron microscopy.

We attempted to differentiate further these two types of cap by testing the effects of inhibitors of active cell movements on

cap formation. These results, obtained by immunofluorescence, are summarised in Table 2. Sodium azide, which may suppress cell movements by blocking intracellular ATP formation¹⁰, inhibited capping of all surface receptors to a similar extent. However, cytochalasin B, which prevents cell locomotion by disrupting cytoplasmic contractile microfilaments¹¹, strongly inhibited capping of surface Ig, con A receptors and Thy-1 alloantigen on spleen cells, but had only a slight inhibitory effect on capping of H-2 alloantigen on spleen cells, and virtually no effect on capping of the other surface receptors. Inhibition of cap formation was readily reversed by incubating treated cells in medium lacking cytochalasin B.

Since cytochalasin B was dissolved in dimethyl sulphoxide (DMSO) before use (100 µg ml⁻¹ in medium 199 containing 10% DMSO), DMSO at the same concentration used in conjunction with cytochalasin B was tested alone for its effects on capping. Results indicate that the differential effect of cytochalasin B on cap formation is not due to the DMSO vehicle. Furthermore, although cytochalasin B has been shown to suppress glucose uptake by cells¹², the observed inhibition

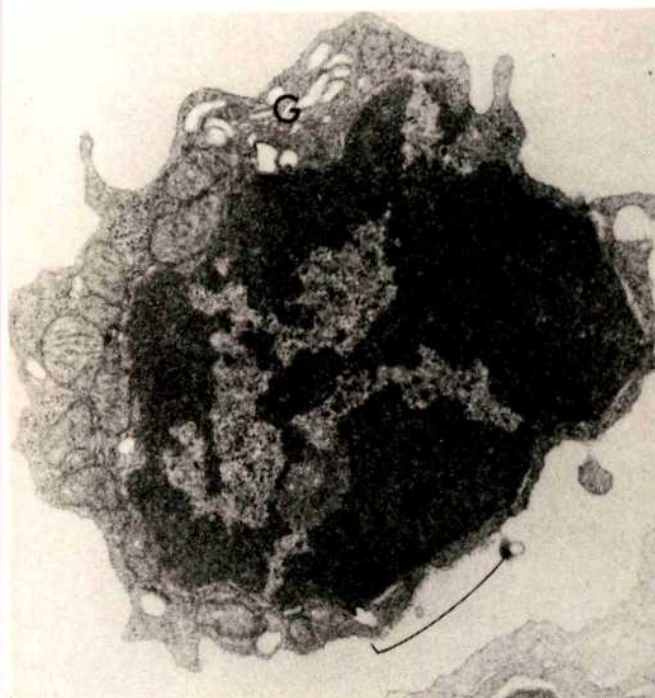


Fig. 2 Section through the approximate centre of a thymus cell, with a cap of TL alloantigen, labelled with anti-TL alloantiserum, hybrid anti-mouse IgG/anti-ferritin antibody, and ferritin, directly opposite the Golgi region (G). The cap of ferritin is marked by a bracket. No internalisation of label is evident. (×17,100.)

Table 2 Effect of Inhibitors of Active Cell Movements on Cap Formation

Receptor	Cell	% Inhibition of capping*				
		Sodium azide (10^{-1} M)	Cytochalasin B (5×10^{-4} M)	DMSO (10^{-1} M)	Vincristine (5×10^{-4} M)	Vinblastine (5×10^{-5} M)
Surface immunoglobulin	Spleen	75	57	7	3	4
Con A receptors	Spleen	86	81	3	0	2
H-2 alloantigen	Thymus	80	7	6	2	0
	Spleen	71	15	8	6	5
Thy-1 alloantigen	Thymus	87	0	2	0	4
	Spleen	86	54	4	5	4
TL alloantigen	Thymus	86	6	6	1	0

* Cells were incubated with the appropriate inhibitor for 15 min at 37° C before immunofluorescence labelling in the continuous presence of the inhibitor. Inhibitors were used at the maximum concentrations at which 80% or more of the treated cells remained viable when labelling was completed. Each figure represents an average of three or more determinations.

of capping is apparently not due to impaired glucose uptake, since labelling of cells in medium lacking glucose had no effect on capping of surface receptors on either thymus or spleen cells.

Vincristine and vinblastine, two anti-mitotic alkaloids which precipitate microtubule proteins¹³, have no significant inhibitory effect on either type of cap formation (Table 2). Colchicine, colcemid and strychnine, drugs which have related effects on cellular microtubules¹⁴, also had no effect on either type of capping when used at a concentration (5×10^{-4} M) at which 80% or more of the treated cells remain viable.

The sensitivity of capping over the Golgi region of spleen cells to cytochalasin B indicates that this type of cap may form as a result of active cell movements mediated by a system of cytoplasmic contractile microfilaments. Capping opposite the Golgi region of thymus cells apparently does not result from the activity of contractile microfilaments or microtubules. Nevertheless, inhibition of this type of capping by sodium azide suggests that some sort of active cell movements is involved. Capping over the Golgi region and extensive endocytosis of capped label may require cell movements controlled by a system of contractile microfilaments, with capping opposite the Golgi region occurring in the absence, or during inactivity, of such a system. The lack of polarity, and relative insensitivity to cytochalasin B, of H-2 alloantigen capping on spleen cells may, however, indicate that there are additional factors involved in determining the cellular site of cap formation.

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T cell factor which can replace T cells in vivo

THE mechanism whereby thymus-derived (T) and bone marrow or bursa-derived (B) lymphocytes cooperate in the induction of an antibody response is of great importance in immunology. It has been suggested that T cells concentrate antigen by their surface receptors to present a multideterminant antigen array directly to B cells^{1,2}; or that T cells secrete a special class of cooperating antibody (IgX) for this purpose, which could specifically present antigen at the surface of a third cell, the macrophage^{3,4}; or that the product of recognition of antigen by T cells has some non-specific mitogenic or stimulatory effect on B cells^{5,6}. Recent work with *in vitro* systems has indicated that various factors produced by T cells, either after contact with a specific antigen or following stimulation by allogeneic cells, can replace the requirement for T cells in antibody formation⁷⁻¹⁴.

Here, I describe the preparation of a T cell factor capable of totally replacing T cells *in vivo* in the induction of an antibody response to a highly thymus dependent synthetic polypeptide antigen, (T,G)-A--L. The factor is prepared *in vitro* by incubation of primed or 'educated' mouse T cells with antigen for 6-8 h; cells are then removed by centrifugation and the supernatant containing the factor is mixed with

TABLE 1 Activity of a T cell factor *in vivo*

Matter transferred into irradiated recipients	Antigen	PFC (\pm s.e.)
B cells*	(T, G)-A--L	124 (\pm 28)
B cells + normal T cells†	(T, G)-A--L	4,360 (\pm 320)
B cells + T cells educated to (T, G)-A--L‡	(T, G)-A--L	5,100 (\pm 364)
B cells + supernatant produced by T cells educated to (T, G)-A--L§	(T, G)-A--L	4,850 (\pm 260)

* 10^7 bone marrow cells.

† 10^8 thymocytes.

‡ 1 spleen equivalent (about 5×10^6 cells).

§ Produced by 1 spleen equivalent educated T cells.

The direct anti-(T, G)-A--L plaque forming cell response in irradiated (850 r.) Balb/c mice 12 days after receiving transfer of B cells, or B cells mixed with normal or educated T cells or a T cell-produced supernatant factor, and (T, G)-A--L. Results as geometric means of 15 mice \pm s.e.

TABLE 2 Conditions of *in vitro* culture for the preparation of a T cell factor

Cells present in culture <i>in vitro</i>	Antigen (<i>in vitro</i>)	PFC(\pm s.e.)*
Normal T cells†	(T, G)-A--L	116(\pm 38)
T cells educated to sheep red blood cells‡	(T, G)-A--L	84(\pm 16)
T cells educated to (T, G)-A--L‡	—	204(\pm 42)
T cells educated to (T, G)-A--L	Sheep RBC	213(\pm 26)
T cells educated to (T, G)-A--L	(T, G)-A--L	4,200(\pm 480)

† 1 thymus equivalent per ml medium.

‡ 2 spleen equivalents per ml medium.

* Direct anti-(T, G)-A--L PFC measured 12 d after transfer of supernatant with 10^7 bone marrow cells and $10 \mu\text{g}$ (T, G)-A--L into irradiated recipients.The direct anti-(T, G)-A--L plaque forming cell response in irradiated (850 r.) Balb/c mice 12 days after receiving transfer of syngeneic B cells mixed with (T, G)-A--L and supernatants produced *in vitro* by different combinations of normal or educated T cells and antigen. Results as geometric means of 15 mice \pm s.e.

normal bone marrow cells and antigen and transferred into lethally irradiated recipients. Under these conditions antibody-forming cells are produced in the spleens of the recipients in numbers identical with those produced by mixtures of T cells, B cells and antigen. Moreover, the T cell factor has been shown to be completely specific for the inducing antigen.

Experiments used Balb/c mice, bred at the Department of Pathology, Cambridge. The antigen was the multichain synthetic polypeptide poly-(Tyr, Glu)-poly-DL Ala--poly-Lys, abbreviated as (T,G)-A--L, and was the gift of Dr Edna Mozes.

T cells were 'educated' or primed to (T,G)-A--L by transfer of 10^8 Balb/c thymocytes into lethally irradiated (850 rad) syngeneic recipients, followed 1 d later by injection of $10 \mu\text{g}$ (T,G)-A--L in complete Freund's adjuvant. The spleens of these recipients, containing the educated T cells, were removed 7 d later, and contained approximately 5×10^6 viable lymphocytes per spleen by the criterion of trypan blue exclusion. Cell suspensions from the spleens were prepared in minimal Eagle's medium (Flow) with no further additions of protein or nutriment, with 10 spleen equivalents of cells per 5 ml of medium. (T,G)-A--L to a final concentration of $1 \mu\text{g}$ per ml was added and the cells incubated in small Petri dishes (Sterilin P 122, 50 mm \times 13 mm) at 37°C , under an atmosphere of 10% CO_2 . The type of Petri dish used is important, since dishes of different grade and manufacture (Falcon Tissue Culture Dishes) gave very unsatisfactory results.

Incubation was continued for 6–8 h, after which the cell suspensions were transferred to a centrifuge tube and the cells spun down. The cell-free supernatant was tested for its ability to cooperate with B cells by mixing with bone marrow

cells and antigen and transferring into lethally irradiated syngeneic recipients. Each recipient received supernatant equivalent to 1 spleen of educated T cells, 10^7 bone marrow cells, and $10 \mu\text{g}$ of (T,G)-A--L dissolved in saline, inoculated into a tail vein. Suitable controls were included as described below.

Twelve days later the spleens of these animals were removed and direct plaque-forming cells (PFC) to (T,G)-A--L determined. For the plaque assay, sheep red blood cells were coated with (T,G)-A--L by mixing equal volumes of packed sheep erythrocytes, chromic chloride (10 mg ml^{-1}), and (T,G)-A--L (2 mg ml^{-1}). After 5 min at room temperature the coated cells were spun down and washed three times. The results shown in the tables are geometric means \pm s.e. with 15 animals per group.

Table 1 shows that the supernatant produced by T cells educated to (T,G)-A--L was able to cooperate with B cells when transferred together into irradiated recipients. The level of the plaque forming response was very much higher than that given by B cells alone, and not significantly different from the response of B cells and 10^8 normal or 5×10^6 educated T cells. Thus, after a relatively short period of incubation with antigen *in vitro*, educated T cells released a factor or factors capable of replacing T cells in a cooperation-dependent antibody response *in vivo*. The conditions required for production of the factor *in vitro* are shown in Table 2. There was an absolute requirement both for educated T cells and the inducing antigen. Neither normal thymocytes, nor T cells primed against a non-cross-reacting antigen (sheep red blood cells) released any significant cooperating activity when incubated *in vitro* with (T,G)-A--L; and T cells educated specifically to (T,G)-A--L only released factor in the presence of (T,G)-A--L.

The specificity of action of the supernatant factor produced to (T,G)-A--L was investigated by transfer together with B cells and a non-cross-reacting thymus-dependent antigen, sheep red blood cells. Table 3 shows that the enhancing activity of the supernatant was specific for (T,G)-A--L and was completely without effect on the response to sheep red blood cells.

The possibility of contamination of educated T cells by B cells must be considered. No anti-(T,G)-A--L PFC were present in the educated T cell preparations, and no amount of conventional anti-(T,G)-A--L antibody tested could replace the requirement for T cells or T-cell factor *in vivo* (personal observation). To demonstrate conclusively that T cells were responsible for the production of the factor, educated T cells were treated with mouse anti- θ serum and complement before culture with antigen *in vitro*. (Anti- θ serum, raised in AKR mice to CBA thymocytes, was the gift of Dr A. Munro.) After treatment with anti- θ serum (or normal mouse serum as control) at a dilution of 1:2 for 30 min, the educated T cell preparation was washed and treated in the cold with guinea pig complement, previously

TABLE 3 The specificity *in vivo* of a T cell factor produced *in vitro* to (T, G)-A--L

Matter transferred into irradiated recipients	Antigen	PFC (\pm s.e.)	
		Anti-SRBC	Anti-(T, G)-A--L
B cells*	SRBC	130 (\pm 33)	
B cells + normal T cells†	SRBC	2,561 (\pm 210)	
B cells + T cells educated to (T, G)-A--L‡	SRBC	116 (\pm 48)	
B cells + supernatant produced by T cells educated to (T, G)-A--L§	SRBC	84 (\pm 26)	
B cells	(T, G)-A--L		250 (\pm 46)
B cells + supernatant produced by T cells educated to (T, G)-A--L	(T, G)-A--L		3,970 (\pm 384)

* 10^7 bone marrow cells.† 10^8 thymocytes.‡ 1 spleen equivalent (about 5×10^6 cells).

§ Produced by 1 spleen equivalent educated T cells.

The direct anti-sheep red blood cell (SRBC) and anti-(T, G)-A--L plaque forming cell responses in irradiated (850 rad) Balb/c mice 12 d after receiving transfer of different combinations of syngeneic B cells, antigen, and either T cells or a supernatant factor produced *in vitro* by T cells educated to (T, G)-A--L. Results as geometric means of 15 mice \pm s.e.

TABLE 4 Effect of anti- θ serum on the ability of educated T cells to produce cooperating factor

Treatment of educated T cells before culture <i>in vitro</i>	Antigen	PFC(\pm s.e.)
None	(T, G)-A--L	3,812(\pm 486)
Normal mouse serum and complement	(T, G)-A--L	2,940(\pm 260)
Anti- θ serum and complement	(T, G)-A--L	282(\pm 106)

T cells educated to (T, G)-A--L were treated with anti- θ serum and complement, or normal mouse serum and complement, before culture with (T, G)-A--L *in vitro*. The factors produced by the surviving cells cultured with (T, G)-A--L *in vitro* were transferred together with B cells and (T, G)-A--L into irradiated Balb/c recipients. The results show the direct anti-(T, G)-A--L plaque forming cells 12 d after transfer. Results as geometric means of $15 \pm$ s.e.

absorbed with agarose and at a dilution of 1:10. Dead cells were subsequently removed by passage through a cotton wool column in low ionic strength buffer after the method of Von Boehmer and Shortman¹⁵. The effluent cells were restored to normal buffer conditions and cultured *in vitro* with antigen for 8 h. Table 4 shows that cells treated with anti- θ serum and complement were unable to produce factor to (T, G)-A--L *in vitro*, whereas controls treated with normal mouse serum and complement showed undiminished ability to produce the active factor.

The cooperating factor described can be compared with that described by Feldmann and coworkers^{4,7}, which is produced by activated T cells *in vitro*, is active in collaborating with B cells *in vitro*, and is also antigen-specific in its induction and action. Its *in vivo* properties have not been reported. On the other hand, there is a clear contrast with various non-specific enhancing factors produced by T cells in response to to allogeneic stimuli^{8-10,12,13} or other strong antigenic stimuli¹⁴.

It is possible that under physiological conditions *in vivo*, the joint action of two factors is required to initiate an antibody response in B cells. The first would be a T-cell product endowed with specificity—presumably through an antibody-like combining site—which could present the antigen in a multivalent fashion to B cells, for example by effectively polymerising antigen at the macrophage surface^{3,4}. The high strength of binding to B cells achieved thereby would be one prerequisite for effective antigen recognition by B cells and for B cell triggering. The other requirement would be a non-specific mitogenic stimulus provided by a second T-cell product, needed to send the B cell into proliferation. An excess of the latter product—as produced by allogeneic stimuli (the 'allogeneic effect')^{5,6,18}—could override the requirement for the former and for multivalent binding of antigen to B cells, by providing unusually high mitogenic stimulation. Thymus-independent antigens would be expected to possess multiple antigenic determinants of the same specificity, and in addition to have an independent mitogenic effect on B cells^{16,17}, thus circumventing T cells altogether.

Studies are in progress to identify the factor described here and in particular to determine whether it comprises more than one active component.

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Demonstration of suppressor T cells in a population of 'educated' T cells

THE regulation of the immune response, in the sense of the limitation of its extent, can probably be achieved in two main ways. The first, and more extensively studied, is the feedback effect of the antibody end-product, brought about either by the removal of antigen, or by the blockage of antigen recognition by antigen-sensitive cells¹. A second possibility suggested more recently is that certain regulatory cells exist which can exert an actively suppressive influence on immune responses². Since they are believed to be among the thymus-derived or T cell population, they may be termed suppressor T cells. Evidence for their existence includes the enhancement of certain responses by anti-lymphocyte serum³⁻⁵, the suppressive influence of graft-versus-host reactions^{6,7}, and recent observations on antigenic competition^{8,9}, infectious tolerance¹⁰, and allotype suppression¹¹.

TABLE 1 Cooperative activity *in vivo* of (T, G)-A--L-educated T cells, before and after culture *in vitro* with (T, G)-A--L

Cells transferred into irradiated recipients	Antigen (in vivo)	PFC \pm s.e.
B cells*	(T, G)-A--L	186 \pm 28
B cells + normal T cells†	(T, G)-A--L	3972 \pm 416
B cells + (T, G)-A--L-educated T cells, before culture <i>in vitro</i> †	(T, G)-A--L	5621 \pm 403
B cells + (T, G)-A--L-educated T cells, after culture <i>in vitro</i> without antigen.	(T, G)-A--L	4214 \pm 318
B cells + (T, G)-A--L-educated T cells, after culture <i>in vitro</i> with (T, G)-A--L	(T, G)-A--L	0

* 10^7 bone marrow cells.

† 10^6 thymocytes.

‡ 5×10^6 viable cells.

The direct anti-(T, G)-A--L plaque forming cell (PFC) responses in irradiated Balb/c mice 12 d after receiving transfer of B cells, or B cells mixed with normal or (T, G)-A--L-educated T cells, and (T, G)-A--L. The treatment of the educated T cells before transfer is indicated. Results as geometric means of 15 mice \pm s.e.

TABLE 2 Suppressive activity of (T, G)-A--L-educated T cells after culture *in vitro* with (T, G)-A--L

Cells transferred into irradiated recipients	Antigen (<i>in vivo</i>)	PFC \pm s.e.
B cells*	(T, G)-A--L	200 \pm 39
B cells + normal T cells†	(T, G)-A--L	4,430 \pm 234
B cells + normal T cells + (T, G)-A--L educated T cells after culture <i>in vitro</i> with (T, G)-A--L‡	(T, G)-A--L	576 \pm 166

* 10^7 bone marrow cells.† 10^8 thymocytes.‡ 5×10^6 viable cells.

The direct anti-(T, G)-A--L PFC responses in irradiated Balb/c mice 12 d after receiving transfer of B cells, or B cells and normal T cells, or B cells, normal T cells and suppressor T cells ((T, G)-A--L-educated T cells after culture with (T, G)-A--L *in vitro*), and (T, G)-A--L. Results as geometric means of 15 mice \pm s.e.

Here I describe a method for demonstrating the presence of suppressor T cell activity in a population of primed or 'educated' T cells. Educated T cells, on culture with antigen *in vitro* for a period of 6–8 h, release a specific cooperating factor which can replace T cells *in vivo*²². The cells remaining in culture at the end of this time have lost the ability to collaborate with bone-marrow-derived (B) cells *in vivo*. Instead, they can suppress the antibody response *in vivo*, as demonstrated by mixing them with normal T cells, B cells and antigen, and transferring into irradiated recipients. Suppression of the immune response by these cells is antigen-dependent, but non-specific in effect. These observations suggest the possible existence of two functional lines of T cells, one cooperative and the other suppressive, and indicates a convenient method for their separation.

The preparation, in Balb/c mice, of T cells educated to the synthetic polypeptide antigen (T,G)-A--L, and the subsequent culture of these cells with antigen *in vitro* has been described in the preceding paper¹². After 6–8 h culture, the cells were spun down and the supernatant removed. The cells were resuspended in cold Eagle's medium, washed twice, and finally resuspended to a concentration of about 5×10^6 viable lymphocytes ml⁻¹. Over the period of *in vitro* culture, a decrease in viability of 15–20% occurred as judged by trypan blue exclusion. The cells were tested for their ability to interact with B cells by transfer together with 10^7 bone marrow cells and (T,G)-A--L (10 μ g in saline per mouse) into irradiated (850 rad) syngeneic recipients. In some experiments, described below, 10^8 normal T cells (thymocytes) were also present in the mixture transferred. Twelve days after transfer, the direct anti-(T,G)-A--L plaque-forming cell (PFC) response in the spleens of these animals was measured as previously described¹². The results in the tables are geometric means of 15 mice \pm s.e.

T cells educated to (T,G)-A--L were able to cooperate with B cells *in vivo* (Table 1), provided they had not been previously cultured with antigen *in vitro*. After incubation with (T,G)-A--L *in vitro* for 6–8 h, the educated T cells could not collaborate with B cells *in vivo* in the response to (T,G)-A--L. Moreover, these cells caused the normal background response observed when B cells and (T,G)-A--L are transferred into irradiated hosts to drop to zero; this was the first suggestion that they were endowed with suppressive properties. This dramatic change in the *in vivo* activity of the educated T cell population did not occur if antigen was omitted from the *in vitro* culture system. Furthermore, the change was antigen-specific, since the cells continued to cooperate normally in the response to non-cross-reacting erythrocyte antigens (personal observation).

To test whether the educated T cells had indeed become actively suppressive after incubation with antigen *in vitro*, they were transferred together with B cells, 10^8 normal T

cells and (T,G)-A--L into irradiated hosts. The usually excellent PFC response produced *in vivo* by B cells and normal T cells was reduced by over 90% by the presence of 5×10^6 educated T cells previously cultured *in vitro* with antigen (Table 2).

The specificity of action of these (T,G)-A--L-induced suppressor T cells was examined by transferring them with normal T and B cells and a non-cross-reacting antigen, sheep red blood cells (SRBC), into irradiated hosts. In one group, (T,G)-A--L was also added. The anti-SRBC response was very significantly reduced by the presence of these cells (Table 3), provided the original educating antigen, (T,G)-A--L was also present. If (T,G)-A--L was omitted, however, then the suppressor T cells had no effect on the response to SRBC. Thus the suppressive effect of these cells was apparently non-specific, but the presence of the specific inducing antigen was necessary to elicit it.

It seems that the following events occur when educated T cells are cultured *in vitro* with antigen for 6–8 h. (i) A specific cooperative factor is released which collaborates with B cells *in vivo* in the induction of an antibody response to the educating antigen, as described in the preceding report¹². (ii) The cells lose the normal ability of educated T cells to collaborate with B cells *in vivo* in the response to the educating antigen. (iii) The cells display a potent suppressive influence on B cells and on mixtures of normal T and B cells *in vivo*. In contrast to the specific cooperative activity of educated T cells or the T-cell factor, the suppressive activity is non-specific in its effects, although the original educating antigen must be present for non-specific suppression to occur.

These results suggest the conclusion that a primed T-cell population contains two functionally distinct groups of cells, one of which is specifically cooperative, while the other is non-specifically suppressive. Brief *in vitro* culture stimulates the cooperative T cell population to release its cooperative factors, but also renders it apparently exhausted at the end of 8 h, although this exhaustion might be only temporary. The suppressive T cell population, on the other hand, is not exhausted by this *in vitro* incubation, and its activity is therefore seen to the full when the cells are transferred with B cells *in vivo*. It seems likely that the suppressor T cells release non-specific suppressive substances shortly after stimulation by antigen *in vivo*, as judged by the efficiency with which the response given by normal T and B cells to SRBC was suppressed by (T,G)-A--L-induced suppressor T cells and (T,G)-A--L. It is also probable that the suppressor T cells constitute an important physiological control mechanism *in vivo*, here demonstrated in the antibody response, but most

TABLE 3 Specificity of suppressor T cell activity: ability of (T, G)-A--L-induced suppressor T cells to inhibit the response to SRBC *in vivo*

Cells transferred into irradiated recipients	Antigens (<i>in vivo</i>)	PFC \pm s.e. (anti-SRBC)
B cells*	SRBC	328 \pm 108
B cells + normal T cells†	SRBC	4,860 \pm 411
B cells + normal T cells + (T, G)-A--L-induced suppressor T cells‡	SRBC	5,640 \pm 384
B cells + normal T cells + (T, G)-A--L-induced suppressor T cells	SRBC + (T, G)-A--L	844 \pm 110

* 10^7 bone marrow cells.† 10^8 thymocytes.‡ 5×10^6 viable cells.

The direct anti-SRBC response in irradiated Balb/c mice 10 d after receiving transfer of B cells, or B cells and normal T cells, or B cells, normal T cells and (T, G)-A--L-induced suppressor T cells, and antigen. Suppressor T cells were prepared by incubation of T cells educated to (T, G)-A--L with (T, G)-A--L *in vitro* for 8 h. Results as geometric means of 15 mice \pm s.e.

likely applying to cell mediated immunity also¹³. Other suppressive phenomena, including some forms of antigenic competition^{8,9}, infectious tolerance¹⁰ and the graft-versus-host reaction^{6,7}, may also be mediated by the same T cell subpopulation. The system described here provides a method for the functional separation of the cooperative and suppressive T cell lines, and should open the way to further clarification of their properties.

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Cyclic GMP Response *in vivo* to Cholinergic Stimulation of Gastric Mucosa

INTEREST in cyclic guanosine 3',5'-monophosphate (cGMP) has been stimulated by the demonstration that its sister nucleotide, cyclic adenosine 3',5'-monophosphate (cAMP), is an intracellular mediator or 'second messenger' for many hormones, including noradrenaline, the neurohormone of the sympathetic nervous system¹. Reports concerning the possible implication of cAMP in gastric secretion in mammals are conflicting. The infusion of histamine increases cAMP in canine gastric juice² but does not stimulate adenylate cyclase activity of canine gastric mucosa³. A review of the subject has led to no consensus of opinion⁴.

An association of cholinergic stimuli with increased tissue cGMP has been described in several organs. Addition of acetylcholine (ACh) *in vitro* increased cGMP in rat heart and liver, rabbit brain, dog thyroid and guinea pig ileum⁵⁻⁸. Mouse brain showed an increase of cGMP with infusion of a cholinergic agonist but not after pretreatment with atropine⁹. Isolated strips of rat gastric fundus contracted when exposed to cGMP¹⁰.

To investigate the possibility that cGMP might be a mediator for cholinergic nervous stimuli in the stomach, an *in vivo* preparation making possible the direct stimulation of the vagus nerves was devised. Acid secretion was demonstrated by measurement of the pH of the gastric mucosal surface. Strips of gastric mucosa (dissected free of the underlying muscularis) were removed from (barbiturate)-anaesthetised adult mongrel dogs and instantly frozen in liquid nitrogen immediately before and after various experimental manipulations. The separation from the blood supply before completion of freezing never exceeded 5 s. The mucosal samples (1 to 2 g) were extracted with trichloroacetic acid and fractionated on a formate column. Cyclic GMP was assayed by radioimmunoassay^{11,12}. The

specificity of the assay was verified by demonstrating the absence of cross reactivity with cAMP and the major mono-, di-, and tri-phosphate nucleotides. Reproducibility was within 5%. Sensitivity was less than 2 pmol cGMP per g tissue. Known amounts of cGMP added to one of two identical samples were quantitatively recovered. ³H-cGMP was used as a recovery standard for the extraction process. All assays were run in triplicate.

Sham operations produced no change in mucosal cGMP content. Results of the various procedures are presented in Table 1. Electrical stimulation (30 pulses (10 ms, 22 V) s⁻¹ for 1 min) of the distal thoracic vagus nerves cut just above the diaphragm more than doubled mucosal cGMP in the fundus of the stomach but did not change antral mucosal cGMP. Pretreatment with intravenous atropine (0.2 mg kg⁻¹), a cholinergic antagonist, prevented the increase in fundic mucosal cGMP.

Table 1 Cyclic GMP Content of Canine Gastric Mucosa

	Fundus			Antrum		
	(pmol g ⁻¹)	N	P	(pmol g ⁻¹)	N	P
Baseline	28±2			49±4		
Vagal stimulation *	66±9	8	<0.001	45±5	5	<0.2
Baseline after atropine	39±5			49±8		
Vagal stimulation after atropine	35±6	4	>0.3	36±1	2	>0.2
Baseline	58±1			57±3		
ACh infusion *	104±5	4	<0.001	58±6	3	>0.5
Baseline	30±5			40±2		
Insulin infusion *	53±10	4	<0.02	37±6	4	>0.5
Baseline	35±4			42±7		
Insulin infusion, vagi cut	31±4	4	>0.2	37±5	3	>0.1
Baseline	30±2					
Pentagastrin infusion *	29±2	4	>0.5			
Baseline				72±6		
Vagal stimulation, fundus out				69±5	4	>0.5

* Acid secretion (reduced gastric mucosal pH).

Infusion of ACh iodide (0.05 mg in 1 min) into the coeliac artery produced a two-fold increase in fundic mucosal cGMP and no change in the antrum, duplicating the results of vagal stimulation. Cannulation of the coeliac artery for infusion was preceded by dissection of the sympathetic-rich coeliac plexus, which may account for an increase in baseline mucosal cGMP in these experiments. Vagal stimulation achieved by hypoglycaemia after intravenous injection (0.6 units kg⁻¹ in a bolus) of crystalline zinc insulin (blood sugar below 50 mg per 100 ml in all dogs) caused a nearly two-fold increase in fundic cGMP with no change in the antrum. Vagus section eliminated the fundic cGMP response to insulin.

Because changes in fundic mucosal cGMP could result from release of the hormone gastrin from the antrum in response to vagal stimulation, the effect of pentagastrin in concentration sufficient to produce acid secretion (1 µg kg⁻¹ over 6 min, infused intravenously) was tested. There was no effect on the cGMP content of the fundic mucosa. Thus, the increase in fundic cGMP with vagal stimulation does not appear to be mediated by gastrin or by the presence of acid in the stomach but is more likely a direct response to cholinergic stimulation.

It seemed possible that the gastric antrum might not be responding to vagal stimulation with a change in cGMP because of the suppressive effects of fundic acid on gastrin-producing cells in the antrum. Therefore, the fundus was resected in four dogs with vagal innervation of the antrum preserved intact, as shown by antral motility when the vagi were stimulated. After removal of this acid-secreting area, vagal stimulation still failed to alter antral mucosal cGMP. The high baseline cGMP in antral mucosa following fundectomy is unexplained.

These data demonstrate an association between several types of cholinergic stimulation of the stomach *in vivo* and an increase in canine fundic mucosal cGMP and suggest that cGMP may serve as a 'second messenger' for ACh, the 'first messenger' of cholinergic stimuli. Cyclic GMP may not perform this function in the gastric antral mucosa, but it is possible that antral mucosal cholinergic receptors are too few in number to raise cGMP to a level within the sensitivity of this assay. The results with gastric fundic mucosa add to the growing body of evidence linking cholinergic stimulation with increases in tissue cGMP.

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Colchicine and Vinblastine Inhibit Fibroblast Aggregation

THE alkaloids colchicine and vinblastine interfere with the assembly of microtubules, yet they also affect several properties of the surface membrane of animal cells. They disorganise an apparent topographical separation between surface areas involved in membrane transport and in phagocytosis in rabbit polymorphonuclear leukocytes¹, inhibit the agglutination of these cells by concanavalin A (ref. 2) and inhibit the concanavalin A-dependent adherence of red blood cells to transformed 3T3 fibroblasts³.

We have now found that these alkaloids also inhibit the spontaneous aggregation of hamster fibroblasts (BHK 21, clone 13 (ref. 4)) which occurs when these cells are shaken in suspension, after dispersal from monolayers by exposure to trypsin in the presence of EDTA (ref. 5). We used experimental conditions and an assay involving the Coulter counter, with which we have previously shown that aggregation is suppressed at low temperature, reversed by low concentrations of proteolytic enzymes⁵, stimulated by neuraminidase⁶ and much reduced in a number of polyoma-transferred derivatives of BHK21 cells⁷.

The inhibition by the alkaloids (Figs 1 and 2) is less complete than can be obtained by treating the cells with high levels

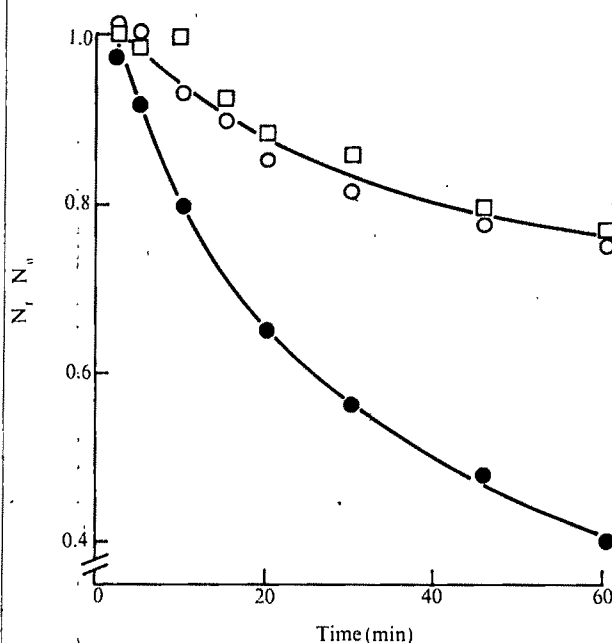


Fig. 1 Inhibition of aggregation of BHK21 cells in suspension by 10^{-4} M colchicine ○ and 10^{-6} M vinblastine □, determined by Coulter counting. These suspensions and control minus alkaloids ● were all aggregated in Hanks' solution buffered with 0.01 M Hepes, pH 7.4. Cells were suspended at an initial density $N_0 = 1.0 \times 10^6$ cells ml^{-1} . Each curve was obtained from 4 ml of suspension incubated in a reciprocating shaker at 37°C in a 10 ml stoppered conical flask. 0.1 ml aliquots were diluted $\times 200$ for counting. The counter measures the total concentration N_t of cells plus cell-clusters.

of trypsin (J. G. E., J. A. Campbell, R. T. R., and M. Vicker, in preparation), and the inhibited cells aggregate more than do polyoma-transformed cells⁷. Vinblastine is effective at much lower concentrations than colchicine, colcemide is intermediate (Fig. 3). Our own unpublished observations show that these concentrations are similar to those required to alter the shape of BHK cells in culture from fibroblast-like to epithelial-like⁸. Lumicolchicine⁹ shows slight inhibition, but only at concentrations 100 times higher than the colchicine from which it was prepared by UV irradiation. The onset of inhibition is fast, occurring within the few minutes resolvable by the assay, and some disaggregation is commonly observed when vinblastine, for example, is added to a partly aggregated cell suspension (Fig. 4). In contrast to these effects on spontaneous aggregation, the agglutination of trypsinised BHK21 cells by concanavalin A seems to be wholly insensitive to the same inhibitors. This is true both for C13 cells (in which case lectin-induced and spontaneous aggregation are superimposed in the assay) and for trypsinised polyoma-transformed BHK21 cells (Fig. 5).

Vinblastine has a very different structure from colchicine, and inhibits aggregation at much lower concentrations. Lumicolchicine, which shares at least some of the non-microtubule effects of colchicine¹⁰, is essentially inactive. It therefore seems more likely that the effects we have observed result from interference with the assembly of microtubules than from some secondary, non-specific effect of the alkaloids on the cell surface. (A remaining problem is that our unpublished observations show that the effect of vinblastine is at least partially reversible, whereas effects of vinblastine on microtubules in some systems are not¹¹.) Microtubules are probably present in trypsinised BHK cells, although Goldman and Follett¹² found them rather sparse and randomly scattered throughout the cytoplasm. It is an intriguing problem how the integrity of microtubules could modify the probability that suspended cells will adhere to one another, and we consider here three possible mechanisms.

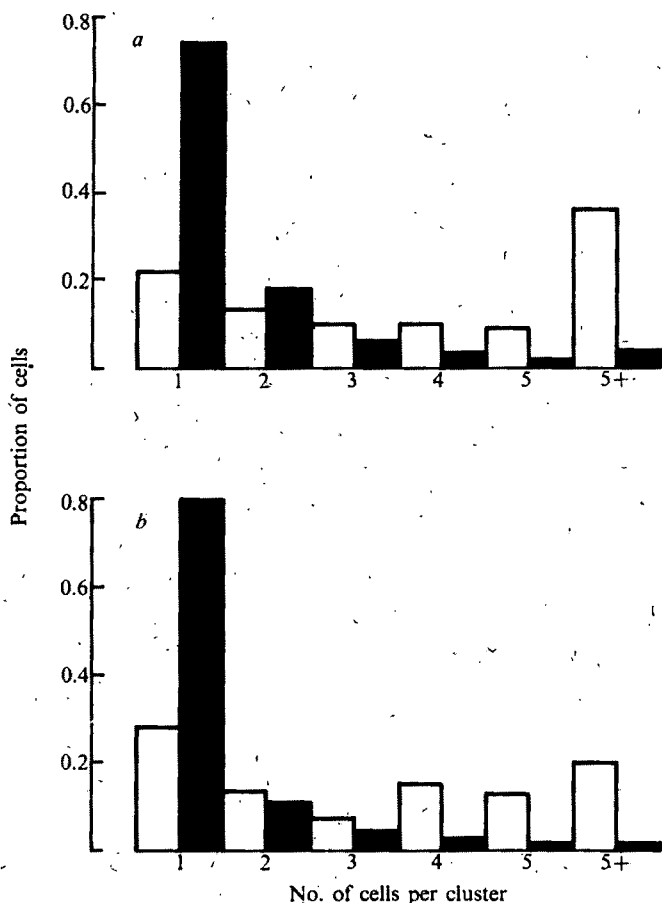


Fig. 2 Inhibition of aggregation by, *a*, 2×10^{-6} M vinblastine and, *b*, 10^{-5} M colchicine, measured by haemocytometry. The ordinate is the fraction of cells found after 45 min incubation in clusters containing the number of cells per cluster shown on the abscissa. Solid figures in the presence of inhibitor, open in absence. About 1,000 cells were counted for each distribution.

First, microtubules may influence the state of convolution of the surface. For example, trypsinised BHK cells are known to possess microvilli¹³ and a relation between fine projections from cell surfaces and cell adhesiveness has often been suggested^{14,15}. The microvilli of BHK cells seem, however, to contain microfilaments rather than microtubules, and are presumably not analogous to the filopodia of mesenchyme cells in the sea-urchin embryo¹⁶, or the axopodia of heliozoans¹⁷. The second mechanism depends on the assumption that the self-adhesiveness of trypsinised BHK cells requires some appropriate arrangement in the surface membrane of 'contact sites' such as those described in *Dictyostelium*¹⁸. We have no evidence for this, beyond a study of the kinetics of aggregation which previously suggested that trypsinised BHK cells behave as though they are adhesive only in patches¹⁹. Berlin has suggested that microtubules can stabilise arrangements of antigenic determinants and other functional elements which would otherwise be capable of rapid lateral diffusion in the membrane¹. This could be achieved by a direct linkage between microtubules and surface elements.

The third mechanism, which we favour at present, is suggested to us by the observations of Vasiliev *et al.* who found that colchicine and vinblastine caused the ruffling activity of fibroblasts locomoting in culture to spread from a restricted area to all parts of the edge of the cells²⁰. Such ruffling has been equated with the insertion, movement and recycling of surface membrane, perhaps by vesicles transported within the cell^{21,22}. Microtubules could be involved in the transport of such surface and perhaps focus its insertion to a restricted part of the cell surface. We propose that cell surface may be

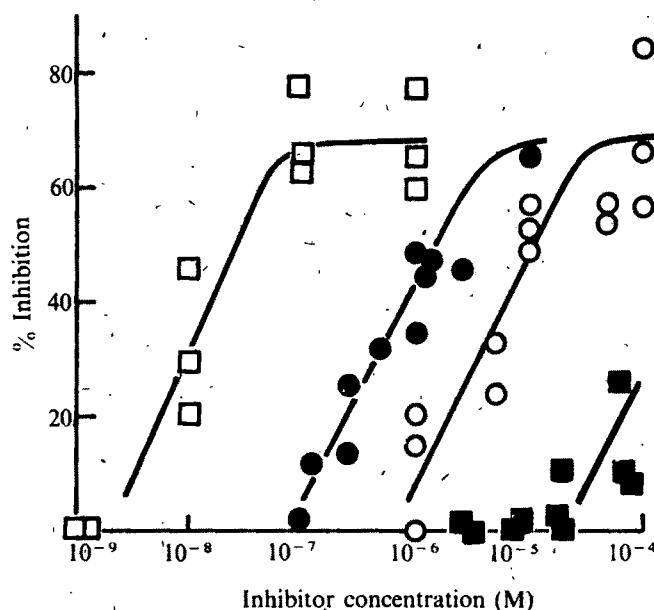


Fig. 3 Concentration dependence of the inhibition of aggregation by vinblastine \square , colcemide \bullet , colchicine \circ , and lumicolchicine \blacksquare . The ordinate is $\frac{N(\text{inhibited}) - N(\text{control})}{N_0 - N(\text{control})} \times 100$, determined after 45 min by Coulter counting as Fig. 1.

adhesive only immediately after undergoing such organised transport, so that aggregation would depend on continued operation of such a cycle. Such a mechanism could account for the sensitivity of aggregation to combined inhibition of glycolysis and respiration which we shall report elsewhere. It is tempting to speculate that a similar surface extrusion phenomenon could be involved in the ADP-dependent aggregation of platelets, which is also sensitive to metabolic inhibitors²³ and to colchicine and vinblastine²⁴.

Whatever the mechanism, if it proves to be true that microtubules can control the distribution of adhesive components in

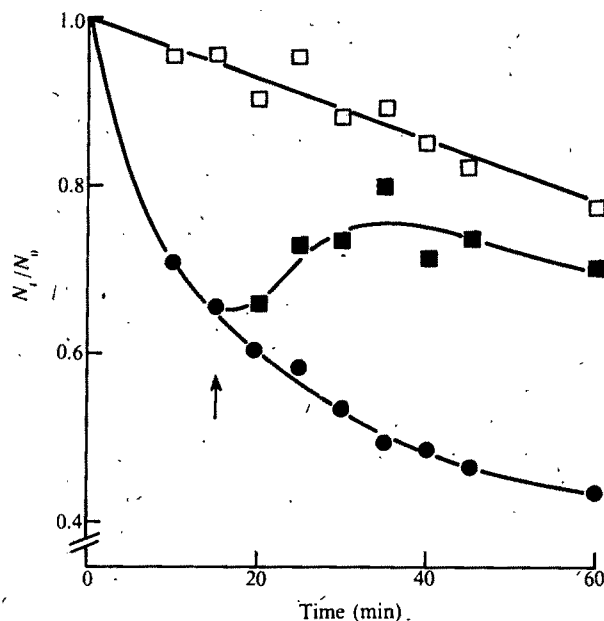


Fig. 4 Kinetics of inhibition of aggregation by 4×10^{-8} M vinblastine, determined by Coulter counting. Vinblastine was added before shaking \square or at 15 min \blacksquare . No addition \bullet .

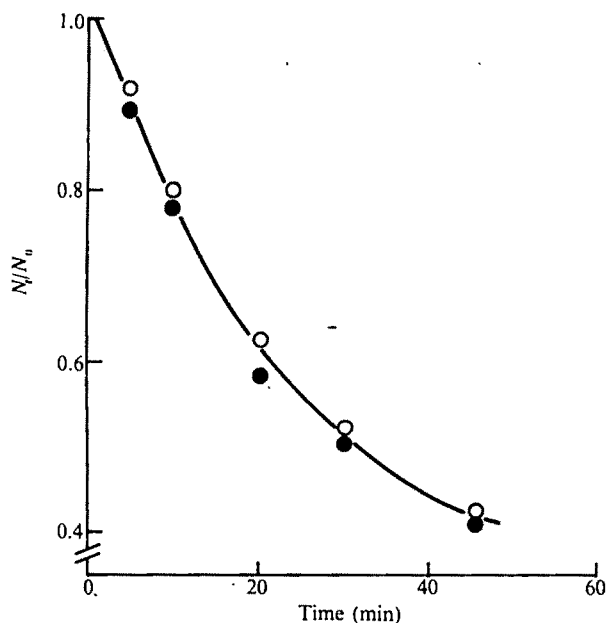


Fig. 5 Lack of inhibition of agglutination of polyoma-transformed cells by concanavalin A ($10 \mu\text{g ml}^{-1}$) by 10^{-4} M colchicine. ○ Con A + colchicine, ● con A only. These cells show a decrease in Coulter count less than 0.1 in the absence of lectin.

cell surfaces, this may be of considerable importance in the organisation of tissues, since it could provide a mechanism whereby cells relate their internal spatial organisation to their orientation with respect to neighbouring cells.

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Explanation of Degree of Correlation of Sibling Generation Times in Animal Cells

SEVERAL methods are available for producing synchronous cultures of mammalian cells^{1,2}, but it is technically impossible to produce a culture which maintains a high degree of synchrony for more than one or two rounds of division because of the highly variable length of the period between successive mitoses from cell to cell. Variability of generation time is also observed in sibling cells formed by the division of a common parent. If cells are able to divide accurately siblings would be expected to be genetically and biochemically identical, and should therefore also have identical generation times but in fact while there is a clear correlation³⁻⁶ it is always imperfect.

In the past this heterogeneity has been implicitly explained in terms of the nature of the sequence of events which a cell must coordinate in order to divide successfully. Because it was considered to be so highly complex, it seemed reasonable to suppose that small differences at each step could accumulate and eventually lead to the great variability in the total intermitotic time observed, even in the case of siblings.

A model of the cell cycle has recently been proposed, however, which positively predicts a high degree of heterogeneity of generation times, but which, in contrast to more conventional models, places much the greatest part of the

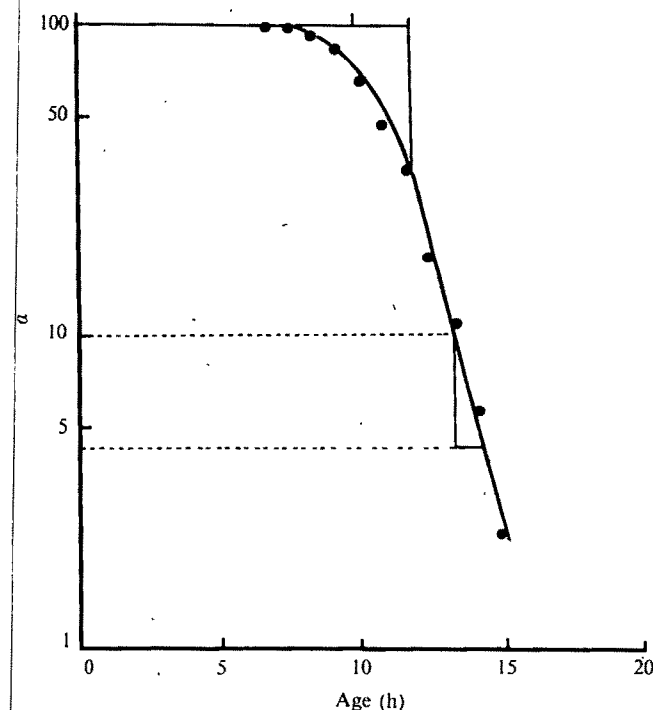


Fig. 1 Semilogarithmic plot of % cells (α) undivided at time t , against t for BHK21/C13 cells. $P = (10 - 4.3)/10 = 0.57 \text{ h}^{-1}$.

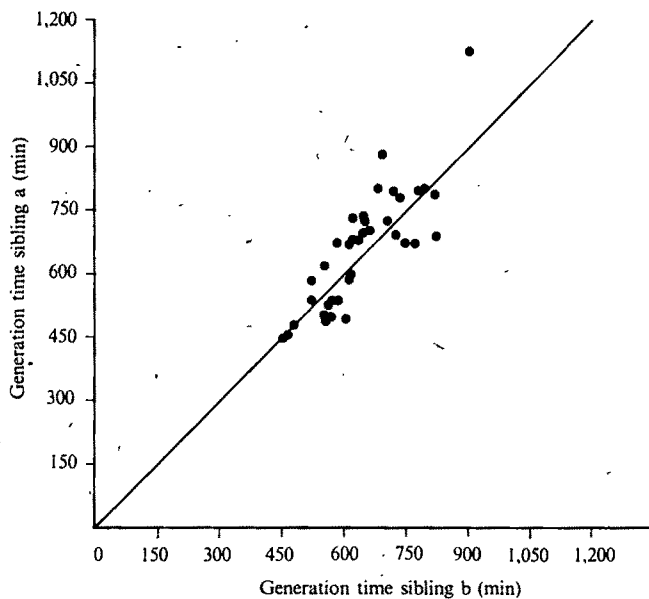


Fig. 2 Correlation between the generation times of siblings for BHK21/C13 cells.

variation at a single point in the cell cycles. According to this theory, proposed in one form by Burns and Tannock⁷, and later by Smith and Martin⁸, there is a sequence of orderly and obligatory events which must be carried out for a successful division. A cell may be either performing this sequence of events, and therefore committed to division (when it is said to be in B phase), or it may be in the alternative condition which Smith and Martin termed the A state.

The A state is regarded as a 'limbo' in which a cell may remain indefinitely without ill effects. A cell in the A state is in no sense committed to division, but is in an unstable state in so far as it may so commit itself at any time, and its probability of doing so is greater the better its physiological condition. A cell in the A state is analogous to a radioactive nucleus in that both may 'decay', the cell into the division sequence of B phase, the radioactive nucleus into its products. Whereas a whole population of cells in the A state will decay at a rate governed by physiological conditions, and a population of radioactive nuclei will decay at a rate governed by the isotopic half-life; it is impossible to state that a specific cell in the A state, or a specific radioactive nucleus is about to decay. The cell's past history (that is, how long it has been in the A state) like that of the radioactive nucleus (how long it has been in existence) does not affect its probability of decaying in the immediate future. Consequently a population of cells in the A state, like a population of radioactive nuclei, will decay with first order kinetics while the time an induced cell spends in the A state is completely unpredictable and therefore by the definition of the word, completely random. It is this random element introduced by the existence of the A state which is said to produce the heterogeneity of intermitotic times in cell populations.

The model makes a number of quantitative predictions which were in good agreement with published observations⁸. The object of this work was to see if it could also provide an adequate explanation of the extent of the correlation of sibling generation times.

BHK21 C13 cells were grown on 30 mm plastic Petri dishes (Nunc, Roskilde) in Dulbecco's modification of Eagles medium supplemented with 10% calf serum. Cells were seeded as sparse cultures the day before filming began, and

gassed with 10% CO₂ in air during incubation at 37° C. Exposures were made at 60 s intervals for 24 h (about two generation times under the conditions used) and the individual intermitotic times measured by frame-by-frame analysis. A cell was said to have entered mitosis when it had completely rounded up before division.

The model predicts a specific type of distribution of intermitotic times. No cell will be able to divide in a time shorter than the length of the necessary sequence of events which makes up B phase (T_b). The remainder of the intermitotic time is entirely attributable to the time spent in the A state, and will therefore have a logarithmic distribution, so that a plot of the fraction of cells (α) having an intermitotic time greater than t against t will show that α remains constant at 100% for a period (which will be equal to T_b) and then decays exponentially just as a population of cells in the A state decays exponentially (because elements of the intermitotic time greater than T_b are spent in the A state). A total of eighty-eight intermitotic times was measured from the BHK cells and is plotted in this form in Fig. 1. The data deviate from the ideal, but for the purpose of discussion this will be assumed to be due to variation in the duration of B phase. On this basis T_b was estimated to be 9.5 ± 0.6 h and the transition probability (defined as the fraction of cells leaving the A state h^{-1}) to be $0.57 h^{-1}$.

The eighty-eight intermitotic times measured included a total of thirty-seven sibling pairs, whose intermitotic times were well correlated ($r=0.85$, $P \ll 0.001$). There was, however, as expected, noticeable scatter from the perfect correlation line (Fig. 2).

The model proposes a two-part cycle, one being the division sequence, and it will be assumed in what follows that this sequence is of identical duration in siblings, that is, that a cell is able to divide its genetic and biochemical capital with total accuracy between its progeny. The other part of the cycle, however, is of essentially random length, and totally independent of genetic or biochemical endowment for an individual cell, and so unrelated in siblings. On these assumptions, cells of cultures which spend a long period in

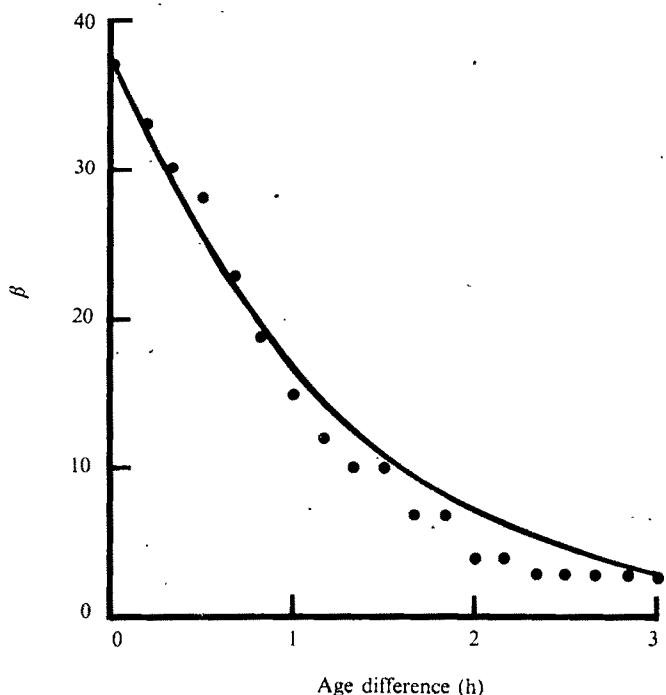


Fig. 3 Semilogarithmic plot of the number of sibling cell pairs (β) with differences in generation time greater than t against t for BHK21. C13 cells, compared with expected line calculated for $P=0.57 h^{-1}$, from Fig. 1. $\beta_t = \beta_0 P^t$, where $\beta_t = \beta$ at time t , P = transition probability.

the A state on average will have, on average, a larger random element in their cycle than cells of cultures which do not, and will therefore also have a greater scatter from the perfect correlation line than rapidly growing cells.

In fact it is possible to make a quantitative prediction concerning the distribution of the differences of intermitotic times of siblings on the grounds that as they represent the differences between times spent in the A state, they will have the same distribution as times spent in the A state. Expressed more rigorously, a transition probability of 0.57 h^{-1} (as here) means that a given cell in the A state has a probability of $(1.00-0.57)$ or 0.43 of not leaving the A state in the next hour. If two cells in the A state are considered at the time when the first one leaves, the second will have a probability

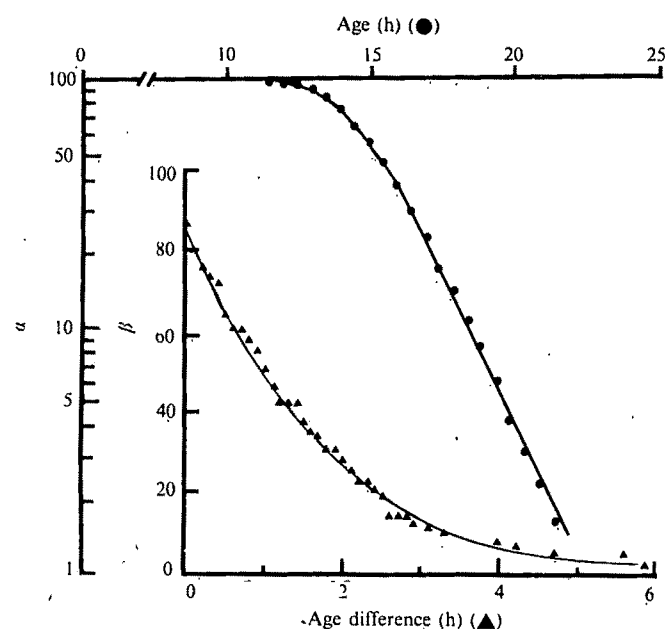


Fig. 4 Semilogarithmic plot of α and β against t for *Euglena gracilis* (data of Cook and Cook³). $P=0.44$.

of 0.43 of remaining in the A state for at least one more hour. Therefore if differences of the intermitotic times of siblings are considered (which have been assumed for the purposes of this treatment to be totally due to differences in the time spent in the A state) a fraction equal to 0.43 of the total will be greater than 1 h . Similar considerations may be applied to different time intervals, and it is consequently possible to deduce a theoretically expected line for the number of sibling pairs (β) whose intermitotic times differ by more than t , against t . Such a line is shown in Fig. 3 for the BHK cells with the actual differences observed. The fit is reasonably good, considering the small numbers involved. Figure 4 shows the plots of α and β for *Euglena gracilis* (data of Cook and Cook³) which involved a far larger number of cells. The agreement between the theoretical and actual distributions is good.

Most current ideas on the cell cycle make no quantitative predictions about the distribution of differences between siblings to be expected, and still less about the relationship between sibling differences and the overall variability of the population. Indeed, there is no *a priori* reason why such a relationship should exist.

In contrast the recently proposed model of the cell cycle^{7,8} does imply such a relationship, in that it should be possible to predict the distribution of the differences between sibling generation times from the distribution of generation times

directly. The data presented here are consistent with the distribution predicted in this way.

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Autosomal recessive mutation in sugar response of *Drosophila*

THE molecular basis of sugar reception in vertebrates and insects still remains undefined. The fact that stimulation with a solution of a pair of sugars exhibits either synergism or an inhibitory effect^{1,2} suggests that multiple receptor sites are involved. Interactions of certain proteins with given sugars are known both in mammals^{3,4} and in insects^{5,6}, but their relation to sugar reception is not clear. Genetic alteration, which has been successful in studies of chemotaxis in a bacterium⁷ and olfaction in an insect^{8,9}, may throw a new light on such a complex system. For genetic dissection of sugar reception, *Drosophila* offers certain advantages. Methods for measurements of its sugar responses are readily available¹⁰, and procedures to isolate its mutants are well established^{9,11}. We shall describe here the first mutant of sugar response in *Drosophila*.

An isogenic line (AA75-3) of *Drosophila melanogaster*¹⁰ was maintained on the usual sucrose-corn-yeast extract medium. Males treated with the mutagen, ethylmethane-sulphonate (EMS)⁹, were mated singly for 4 d with untreated virgin females of the marker strain T5 having genotype *Cy/Pm*; *Ubx/Sb* (*Cy*, *Curly* wings with inversions; *Pm*, *Plum* eye colour; *Ubx*, *Ultrabithorax* halteres with inversions; *Sb*, *Stubble* bristles) with an attached X chromosome, and transferred to be mated with new females of the marker strain T5 for another 4 d. Each F_1 progeny male of *Cy/+*; *Ubx/+* was then backcrossed to three virgins of the marker strain T5. Thus homozygotes, the autosomes of which came from one side of the chromosomal pair of each treated male, were isolated in descendants. From about 1,200 F_1 males, only 100 isogenic lines were obtained (about 300 lines possessing autosomal recessive lethal genes have been maintained with *Cy* or *Ubx*, and others were lost due to the lethal effects of the mutagen).

To detect suspect lines revealing abnormal behaviour, we examined changes in sugar responses of about 100 isogenic lines of flies aged 0-3 d with the preference-aversion test according to Isono *et al.*¹⁰, using a balanced system with the following four stimulants placed in four small glass rings in a Petri dish: distilled water, $5 \times 10^{-2} \text{ M}$ D-fructose, $7 \times 10^{-2} \text{ M}$ D-glucose and $2 \times 10^{-2} \text{ M}$ sucrose each in a 2% agar solution. Concentrations of sugars were determined so as to attract the parent flies (parent strain AA75-3) at a ratio of about 1:1:1:0.2 for fructose, glucose, sucrose and water, respectively. The unbalance of the ratio resulting from changes in responses of flies to any of these three

TABLE 1 Responses of mutant, parent, F₁ and F₂ hybrid flies to sugars

Sugars	Concentration (M)	Number of impulses/0.2 sq*			
		Parent	Mutant	F ₁ hybrid†	F ₂ hybrid‡
Sucrose	10 ⁻²	15.7	13.2§	15.5	14.7
	10 ⁻¹	24.6	26.0	25.6	25.9
	10 ⁻²	19.9	17.8§	19.7	19.7
Maltose	10 ⁻¹	25.2	24.5	25.0	25.4
	10 ⁻²	6.7	6.1	6.6	6.7
	10 ⁻¹	15.7	15.0	14.9	15.9
D-Fructose	10 ⁻²	10.6	3.4¶	9.6	6.4
	10 ⁻¹	19.6	14.5¶	19.4	17.5

* Average number of impulses originating in sugar receptors of labellar single chemosensory hairs of *Drosophila* flies aged 0–2 d during 0.2–0.4 s after initiation of stimulations by the 'tip recording method'. Number of stimulations was about 2,000 with 267 hairs of 89 flies (45♂, 44♀).

† Coming from two reciprocal classes of single crossings of mutant with parent.

‡ Coming from two reciprocal classes of single crossings of F₁ hybrid with mutant.

§ ¶ Significantly different from parent (§, $P < 0.05$; ¶, $P < 0.01$).

|| Significantly different both from parent and mutant ($P < 0.05$).

sugars could be seen at once. Of about 100 lines examined, one line of flies (126B04) showed a marked decrease in response to glucose, giving an average ratio of about 1.2:0.3:1.1:0.2 for fructose, glucose, sucrose and water, respectively.

Such behavioural changes seem to be a consequence of functional modifications in the chemosensory hairs. To define the regions where modifications were involved, nerve responses of labellar chemosensory hairs of the mutant flies (line 126B04) were compared with those of the parent by the 'tip recording method' used in studies of contact chemoreception in dipterous insects^{10,12}. The nerve responses of flies aged 0–2 d were recorded from the tips of three particular type-L labellar single hairs with a glass capillary filled with sugar dissolved in 10⁻⁴ M NaCl solution, which served as a stimulator and recording electrode, and an indifferent electrode inserted into the back of the head¹⁰. Experiments were done at 20° C in a relative humidity range from 60 to 70.

Column 3 and 4 of Table 1 show the average number of impulses originating in the sugar receptors of single hairs of both mutant and parent flies during 0.2–0.4 s after the initiation of the stimulations by 10⁻¹ and 10⁻² M sucrose, maltose, glucose and fructose. The responses of the mutant flies to glucose are drastically different from those of the parent, the average difference being about 5.1 and 7.2 impulses for 10⁻¹ and 10⁻² M glucose, respectively; whereas, with both 10⁻¹ and 10⁻² M fructose, the mutant flies reveal phenotypes similar to those of the parent. The changes of responses in the mutant flies to both sucrose and maltose seem to be dependent on the mutation present; although the mutant flies responded normally to 10⁻¹ M of those sugars, they revealed reductions of about 2.5 and 2.1 impulses for 10⁻² M of sucrose and maltose, respectively. In addition to these four sugars, the labellar hairs of the mutant flies were completely insensitive to 1 M D-galactose, D-mannose and D-xylose, and 3 × 10⁻¹ M lactose as expected from the results with the parent flies¹⁰. Male flies revealed phenotypes similar to those of females of the same strain in response to stimulations by sucrose, maltose, glucose and fructose (for example, 14.6 and 14.4 impulses, to 10⁻¹ glucose, and 3.7 and 3.1 impulses to 10⁻² M for males and females of the mutant flies, respectively). This indicates that the mutation present can be independent of the sex chromosomes, and probably is in the autosomes.

Column 5 of Table 1 shows the average magnitudes of nerve responses of the 20 F₁ hybrid flies, which came from two reciprocal classes of several single crossings of the mutant flies with the parent. It is evident from this table that the F₁ hybrid flies reveal phenotypes similar to those of the parent in response to the four sugars. No significant

difference in response was recognised between sexes. From the above results, we conclude that the mutation is autosomal recessive.

Column 6 of Table 1 shows the average values of impulses of the 40 F₂ hybrid flies, coming from two reciprocal classes of several single crossings of the F₁ hybrid flies with the mutant. The F₂ hybrid flies revealed phenotypes similar to those of the parent in response to sucrose, maltose and fructose; whereas to both 10⁻¹ and 10⁻² M glucose, the F₂ flies gave responses intermediate to those of the parent and mutant strain. Male F₂ hybrids showed similar magnitudes of response to their females. An attempt to define segregations of the F₂ hybrids between phenotypes of both strains on the basis of their responses to stimulation by 10⁻¹ and 10⁻² M glucose has been unsuccessful because of marked variations within each group of the parent and mutant flies, which deterred us from drawing an arbitrary line within the F₂ hybrid flies.

Although the mutation could not be defined other than that it is an autosomal recessive, the fact that the mutant flies revealed simultaneous modifications in response to glucose, sucrose and maltose, though they showed normal responses to fructose, implies that at least two different receptor sites are involved in labellar chemosensory hairs of *Drosophila*. If further genetical analysis shows that the mutation is governed by a single gene, comparisons of the mutant flies with the parent will lead to isolation and identification of receptor sites in the labellar chemosensory hairs.

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Blood Glucose and Nutritive Antibiotic Activity in Ruminants

NUMEROUS monogastric species respond to the continuous ingestion of small amounts of certain antibiotics by increased growth and improved feed utilisation. The mode of action of this so-called "nutritive effect" is not well understood, although various alterations in the intestinal flora have been observed as well as changes in the intestinal mucosa, indicating improved nutrient absorption.

Ruminants also exhibit growth responses to some antibiotics, but here the mode of action is even less clear. Changes in the rumen metabolites and flora are likely to occur, and hypotheses trying to explain the antibiotic growth response in ruminants are largely based on altered rumen fermentation¹. No consistent changes in rumen or host metabolism have so far been demonstrated, however, which could be related to growth responses.

Leskova and Zucker² have recently reported that the growth response of early weaned calves to the new phospholipid-antibiotic Diumycin (SFI 80.586) was accompanied by elevated blood glucose levels. This antibiotic has a predominantly Gram-positive spectrum and is not active against protozoa. It is not absorbed and therefore its primary action should be confined entirely to the lumen of the intestinal tract. Antibiotic levels of 5 and 10 p.p.m. in the feed were employed, the lower level corresponding to approximately 0.1 mg, or less, per kg of body weight. The calves were fed a complete pelleted feed (maize 40%, oats 15%, alfalfa meal 17%, soya bean meal 25%, minerals and vitamins 3%) with hay.

The results of two experiments are shown in Figs 1 and 2. Similar increases of weight gains and blood glucose levels have been measured in two additional experiments. There were no changes in serum lactate levels. Serum urea concentration decreased significantly in calves receiving the antibiotic, indicating increased protein synthesis³. Diumycin improved feed utilisation in all experiments by 5 to 10%.

Bergman⁴ showed that blood glucose concentration in sheep is closely related to turnover rate of glucose. This implies that the rate of glucose utilisation is dependent on sufficiently high blood glucose levels. It can be assumed that such elevated blood glucose levels also stimulate insulin secretion⁵ and thereby amino acid transfer into cells. These findings would offer an explanation for the growth response to antibiotics in ruminants, which seems, according to general experience, more pronounced when diets with high roughage content are fed. Such diets tend to lead to a relative "glucose deficiency" not

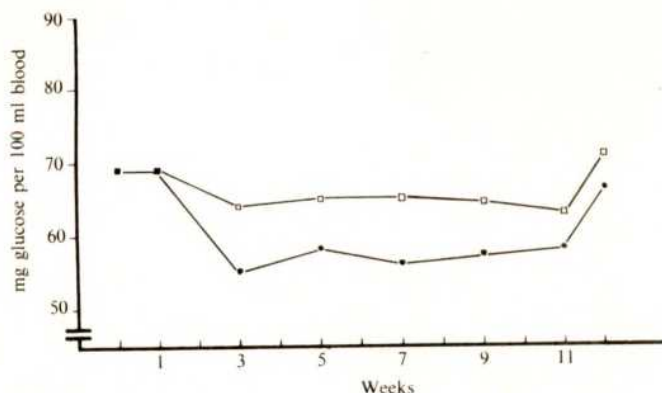


Fig. 1 Effect of Diumycin on growth and blood glucose. Twelve female, individually fed calves (starting weight 68 kg) were subjected to each treatment. Concentrates and hay were given *ad libitum*. With no Diumycin in the feed (●), the average daily gain in body weight was 709 g. With 10 p.p.m. (□) of Diumycin in the feed the average daily gain was 823 g. The arrow indicates the withdrawal of milk replacer.

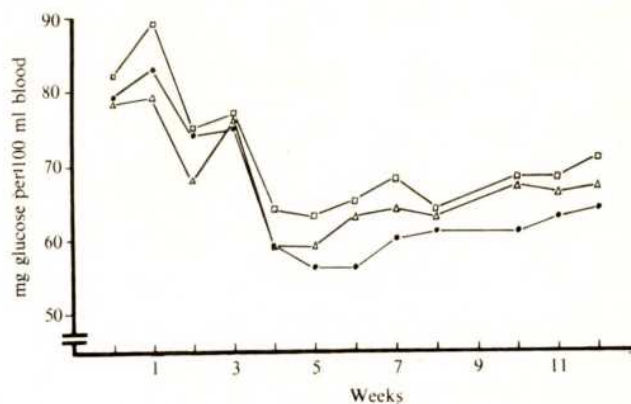


Fig. 2 Relationship between the level of Diumycin in the feed, growth and blood glucose. Sixteen female, individually fed calves (starting weight 61 kg) were subjected to each treatment. Concentrates were fed *ad libitum* but hay was restricted. With no Diumycin in the feed (○), the average daily gain in body-weight was 680 g; with 5 p.p.m. (△). Diumycin in the feed the gain was 748 g and with 10 p.p.m. (□) in the feed the gain was 743 g. The arrow indicates the withdrawal of milk replacer.

only by supplying less preformed glucose, but also by favouring formation of acetic acid rather than propionic acid, which is the main precursor of glucose in ruminants.

We conclude that nutritive antibiotic action in the ruminant is related to elevated blood glucose, which may result from either increased rumen synthesis of propionic acid or decreased degradation of starch and sugars in the rumen.

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Structural Difference between Luminal and Lateral Plasmalemma in Pancreatic Acinar Cells

THE acinar cells of the pancreas, as well as other exocrine protein secreting cells, are highly polarised. Release of secretion products, which occurs by exocytosis (fusion of the secretory granule membrane with the plasmalemma (PM) followed by opening at the point of fusion¹, is limited to the restricted portion of the cell surface facing the secretory lumen. It is still unclear whether this selective localisation of discharge depends on the specific properties of the luminal portion of the PM. Studies carried out in other cell systems have indeed revealed that regions of the PM where specialised functions are located can exhibit distinct characteristics²⁻⁵. But in the pancreas a comparative analysis of the secretory portion of the PM relative to the non-secretory portion has never been reported. By the use of freeze-fracture we have now observed that the luminal PM is different in structure from the remainder of the PM. Rather it is reminiscent of the membrane with which it is functionally associated, the membrane of the zymogen granule (ZG).

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Male albino guinea pigs were starved for 24 h and then killed by a blow on the head. Small pancreas tissue fragments were fixed for 30 min in 1.5% glutaraldehyde and 1% formaldehyde, freshly prepared from paraformaldehyde, in phosphate buffer, pH 7.3, then infiltrated for 3 h in 30% glycerol in 120 mM phosphate buffer, pH 7.3. Samples were frozen by immersion in Freon 22, cooled to -150°C in liquid nitrogen, then freeze-fractured according to the method of Moor and Müllethaler⁶ in a Balzers freeze-etching device. Fracturing temperature was -115°C . Platinum-carbon replicas were washed in Na hypochlorite to remove organic material, then in distilled water and recovered on 200 mesh copper grids. The replicas were examined in a Philips EM 200 electron microscope.

Large areas of both the luminal and lateral PM were exposed by cleavage. Sheets of apical PM are easily identified by the presence of a surrounding continuous network of thread-like structures which, from previous studies, are known to correspond to tight junctions⁷ and by the occurrence of rounded elevations or depressions and finger-shaped structures which are identified as cross-sectioned and tangentially exposed microvilli.

It is well known that the PM is a highly asymmetrical structure⁸. In general, after cleavage, most of the particles are associated with the half of the membrane left frozen to the cytoplasm (the A face). The face of the half membrane left frozen to the extracellular space (the B face) bears fewer particles per unit area. In pancreatic acinar cells the typical A face pattern can be found only in the basal and lateral portions of the PM (including the regions close to the tight junctions and within the chambers delimited by their network). But over the entire luminal area there are far fewer particles per unit area of the A face (Fig. 1). The transition is very sharp across the inner continuous line of the tight junction delimiting the lumen, as can be seen when the fracture plane runs continuously from the lateral regions through the junctional complex to the luminal area (Fig. 1a). On the B face the number of particles is always very small, with no clear difference between the luminal and the lateral portions of the PM (Fig. 1b).

The limiting membranes of the ZG stored within the cells bear many fewer particles per unit area than the membranes of the other cytoplasmic organelles. The distribution is again asymmetrical, with more particles in the half membrane left frozen to the surrounding cytoplasm (A face) (Fig. 1, inserts). Hence, if we look at the particle patterns, the B face of the ZG

membrane resembles the B face of the whole PM while the A face of the ZG membrane looks like the luminal PM A face. Since during exocytosis the A leaflet of the ZG membrane becomes continuous with the A leaflet of the luminal PM and the same occurs with the two B leaflets, we have been led to postulate that the morphological similarity is more than a casual event and might be the expression of a similar biochemical composition. Indeed, our previous studies on isolated cellular membranes of the guinea pig pancreas have shown that the ZG membrane and the PM are similar in lipid composition⁹. Also, at least some of the polypeptides present in the ZG membranes seem to be present also in the PM preparations, even if the polypeptide composition of the latter fraction is much more complex¹⁰. Our PM preparations, however, represent portions of the entire surface, whereas a meaningful comparison should be made with the luminal portion only, which accounts for a small proportion of the total fraction¹¹.

It might be argued that the peculiar structure of the luminal PM could only depend on the continuous insertion of ZG membrane patches. Since ZG membranes contain few particles such insertion could result in a 'dilution' of the typical PM particle density. But if this were the case, one would expect to find, at least in some cells, an uneven distribution of particles over the luminal A face (ranging from areas similar to ZG membranes to areas of the typical PM type) and no sharp transition at the level of the tight junction. Our results were exactly opposite to this. Furthermore, in our experiments the animals were starved and in these conditions the rate of discharge of ZG is known to be very low. We believe therefore that the peculiar structure that we have observed is probably intrinsic to the luminal PM and could be maintained by a selective restriction in membrane mixing occurring in the PM at the level of the tight junction.

Recent turnover experiments in which ZG membrane proteins have found to have much longer half lives relative to the secretory proteins are consistent with the idea that ZG membranes can be reutilised in many secretory cycles¹². Thus, once fused with the luminal PM, they should be somehow reinternalised, either as discrete macromolecules or as intact membrane patches, to be reassembled within the cell. In agreement with this conclusion it has been previously observed that a population of vesicles, possibly retrieved from the luminal PM, appears in pancreatic acinar cells following stimulation of secretion¹³. These results, together with those we report, make it tempting to suggest that the ZG membranes and the luminal portion of the PM might be part of a unique pool of membranes which are continuously shuttled backward and forward between the newly formed ZG and the luminal surface. It is interesting that also in other cellular systems (the toad bladder epithelium and the endothelia^{5,14}) the vesicles known to be able to specific interaction (fusion-fission) with the PM have particle pattern and polarity similar to the portion of PM with which they interact. It therefore seems possible that the phenomena which are at the basis of our observations might not be limited to secretory cells but have a more general occurrence.

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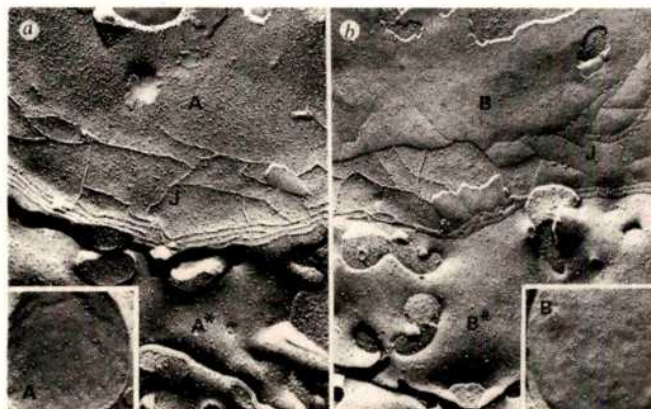


Fig. 1 Freeze-fracture of guinea pig pancreatic acinar cells. The fields illustrate the appearance of large sheets of PM in the regions of transition between the lateral and the luminal (*) portions, and include the tight junctions (J). The face frozen to the cytoplasm is labelled A, that frozen to the extracellular space is labelled B. The inserts illustrate the outer (A) and inner (B) leaflets of the ZG membranes. Shadowing was from the bottom. ($\times 45,000$.)

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Heterotransplantation of human foetal organs to the mouse mutant *nude*

THE mouse mutant *nude* displays thymic aplasia which entails a defect in cell-mediated immune responses. This immune deficiency has been demonstrated by transplantation of normal allogeneic skin and heterogeneic skin from mammalian donors including man, and from avian and reptilian donors¹. The *nude* mutant also accepts transplants of many human malignant tumours, including adenocarcinoma of the colon and rectum, malignant melanoma, squamous cell carcinoma and Burkitt's lymphoma (refs 2-4 and C. O. P., unpublished results). We report here the results of experiments involving transplantation of human foetal organs to the *nude* mouse.

Donors were five 14- to 22-week-old human foetuses, the results of pregnancies terminated by Caesarian *sectio parva*. The reasons for termination are given in Table 1 together with the gestational age and sex. All foetuses were considered normal. Immediately after the section, foetal thymus, lung, myocardium, pancreas, adrenal, kidney, spleen, liver, testis, ovary and spinal cord were transplanted in *nude* mice. The inocula measured about 2 × 1.5 × 1.5 mm and were implanted subcutaneously in the lateral abdominal wall.

Table 1 Reasons for Termination of Pregnancies, Gestational Age and Sex of Human Donor Foetuses

Experiment No.	Indications for Caesarian <i>sectio parva</i>	Gestational age (week)	Sex
1	Social	16	m
2	Neurosis neurasteniformis	14	m
3	Rubella	20	f
4	Social	18	m
5	Rubella	22	f

Recipient *nudes* were the 6-week-old progeny of both sexes of the sixth and seventh cycle of backcross mating with Balb/c mice. Breeding was conducted at the Pathological Anatomical Institute, Kommunehospitalet, Copenhagen¹. Testicular and ovarian transplants were restricted to the corresponding sex, but otherwise no account was taken with sex. Animals were observed daily and transplant growth was noted until death or killing.

Tissues were taken from the implantation sites for histological studies. All were fixed in neutral formalin (apart from testis and ovary tissue where Cleland's fixative was used) embedded in paraffin wax, and 7 µm sections were cut and stained with haematoxylin and eosin and van Gieson-Hansen. Four µm serial sections of testis and ovary transplants were stained with iron haematoxylin using Rowley and Heller's method⁵. Observation times after transplantation and the results are given in Table 2.

Table 2 Results of Transplantation of Human Foetal Tissue to *Nude* Mice

Inoculated tissue	Observation (d)	Fate of graft (accepted/total)
Thymus	19-59	7/13
Lung	13-64	10/12
Myocardium	54-60	0/2
Pancreas	64	1/1
Adrenal	17-39	3/12
Kidney	18-36	2/4
Spleen	21-48	0/7
Liver	17-57	0/10
Testis	23-57	3/5
Ovary	5-33	2/4
Spinal cord	21-57	0/2

Macroscopically, lung, thymus, testis and ovary transplants progressively increased in size. Lung transplant growth was most marked, maximally to 6 × 8 × 9 mm. At autopsy the accepted transplants were well defined, apparently encapsulated in the subcutaneous space, and richly vascularised by regional vessels. When transplants did not take, only sparse yellowish tissue remnants were found. Microscopic examination of accepted organ transplants showed these to be richly supplied by host vessels. There was only a minimal inflammatory reaction consisting of a few mononuclear cells. The histological appearance of these organs was as follows.

The thymus was of normal lobular appearance with some differentiation of cortex and medulla. The epithelial elements in the medulla were further differentiated so that number of Hassall's corpuscles were readily identifiable. The cortical vessels were sheathed by epithelial cells, and the cortex otherwise dominated by lymphocytes with a moderate number of mitotic figures. Reticulum cells and histiocytes were in normal concentrations. Compared with the donor material, the distinction between cortex and medulla was less apparent. An increase in the number of Hassall's corpuscles was noted, but these were of normal appearance (Fig. 1).

The lung showed a domination of markedly dilated alveolar-like structures with a much flattened epithelium. Ducts, the primitive bronchial structures, were clearly seen passing from the alveolar formations. These were lined by one layer of ciliated columnar cells with nuclei placed towards the lumina. Smooth muscle and isolated immature cartilaginous tissue in a mesenchymal stroma were seen in the duct wall together with normal vessels. By comparison with original transplants there was a very marked reduction in mesenchymal tissue elements. (Fig. 2a-c).

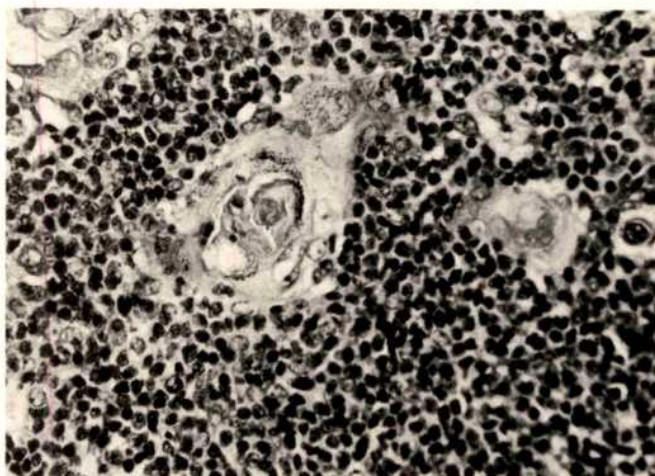


Fig. 1 Human foetal thymus 59 d after transplantation to *nude* mouse. Hassall's corpuscle surrounded by lymphocytes, histiocytes and reticulum cells × 270. Haematoxylin and eosin (HE).



Fig. 2 *a*, Human foetal lung tissue before transplantation (experiment 1). Primitive bronchial structures (ducts) in abundant mesenchymal stroma $\times 270$. *b*, Low power view of same lung tissue 36 d after inoculation in *nude* mouse. The transplant is situated in the subcutaneous space between skin (top) and abdominal muscle layer (bottom). $\times 40$. (HE). *c*, Detail of *b* showing duct surrounded by alveolar-like structures. Note marked reduction in mesenchymal stroma. $\times 242$. (HE).

The pancreas was of normal lobular appearance. Normal acini and small ducts constituted the exocrine elements of the gland, and the islets of Langerhans were normally formed. The histological picture was in good accordance with donor material. In the adrenal, viable tissue comprised only cells of the adult or provisional zone. These were small with round chromatin-rich nuclei which filled most of the cell. The sparse cytoplasm contained few vacuoles. In the foetal zone there was extensive necrosis. No medullary tissue was seen (Fig. 3).

The recovered kidney tissue closely resembled the original transplant. Glomeruli were of variable size, but probably normal in number. Two or three vessel roots were seen with patent lumina and containing erythrocytes. The proportion of

mesangial tissue seemed to be as in the original implant. The visceral and parietal aspects of the Bowman capsules were covered with regular cylindrical epithelium. The capsular spaces seemed to be empty. Tubules were well preserved with an epithelium resembling that of the implant. The proportion of interstitial tissue was increased. This tissue was a loose, connective tissue with many vessels of normal appearance. (Fig. 4 *a, b*).

The general structure of the testis implant was preserved with interstitial tissue containing seminiferous tubules of normal size. The latter contained gonocytes and undifferentiated Sertoli cells. These cells could not be distinguished from normal foetal cells. Some of the interstitial cells resembled fibroblasts while others had some similarity to Leydig cells (Fig. 5).

The stroma of implanted ovaries was of normal appearance and contained a mixed population of oogonia and oocytes. No definite progression in the ovarian development could be demonstrated in the transplants when compared with histology of the tissue before implantation.

At the sites of spleen, liver, myocardium and nervous tissue implantation only necrotic tissue remnants were found with accumulations of macrophages.

Thus the study showed that many human foetal organs (thymus, lung, pancreas, adrenal, kidney, testis and ovary) can be successfully transplanted to the *nude* mouse. Histologically the structure of these organs was essentially preserved, and some development occurred. The appearance of the lung transplants needs a special comment. The dilated alveolar-like structures may be explained by secretion retention dependent on the ectopic lie and by a normal development and differentiation to alveolar-like structures with reduction of mesenchymal elements. This investigation was too small to give any explanation of why some transplants take and others do not, or have not so far.

Other *in vivo* models have been used for human organ transplantation: for example, the chorio-allantoic membrane in the fertilised chicken egg⁶, neonatal rodents⁷ and immunologically tolerant⁸ or immunosuppressed animals⁹. The study organ has generally been human skin, and takes have been achieved for varying periods.

This communication reports the first successful transplantation of human foetal organs to the *nude* mouse. The system seems to offer rich opportunities for the study of growth and differentiation of these organs. Of immediate further interest would be a study of the effects of hormones and carcinogens, as well as toxic, teratogenic, and infectious agents on foetal tissues thus transplanted. The special opportunities which heterotransplanted testicular and ovarian tissue offer will be discussed elsewhere¹⁰. Two important limiting factors need

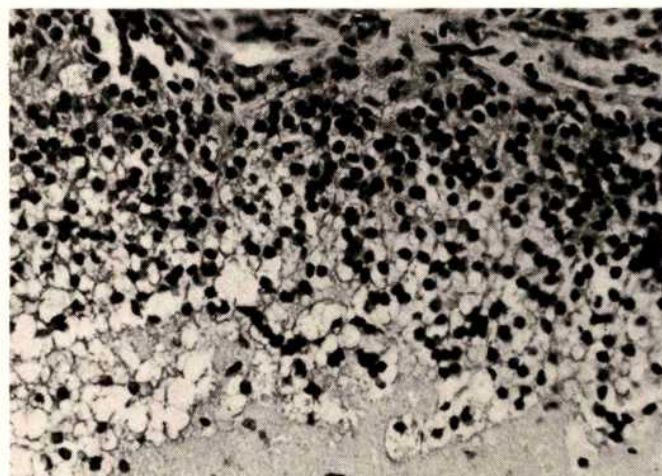


Fig. 3 Human adrenal tissue 28 d after inoculation. Cells from the provisional zone are viable, whereas the foetal zone is necrotic. $\times 270$ (HE).

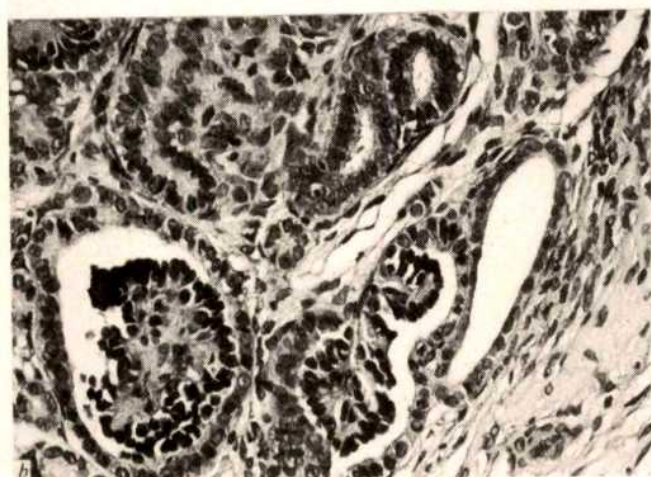
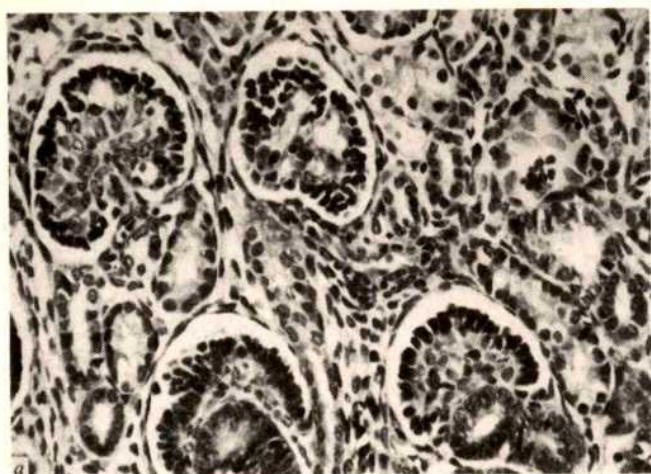


Fig. 4 a, Human foetal kidney (exp. 4) before inoculation. $\times 270$ (HE). b, Same tissue 36 d after inoculation in nude mouse. The primitive glomeruli and tubules closely resemble the donor material $\times 270$ (HE).

however to be mentioned. Donor material is not readily available, and the nude mouse is extremely prone to infection which even under good conventional conditions limits the nude lifespan to 4 or 5 months. We expect that exploitation of strict specific pathogen free or germ-free milieu will largely compensate for the latter difficulty¹.

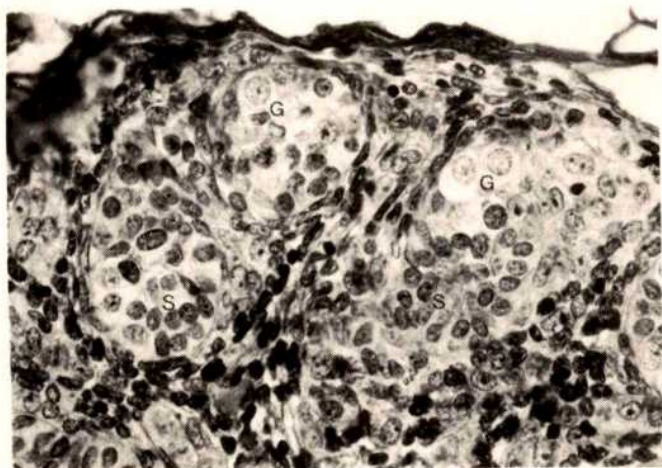


Fig. 5 Human testicular tissue 36 d after implantation. Three seminiferous tubules containing Sertoli cells (S) and gonocytes (G) are shown $\times 340$, iron haematoxylin.

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Efferent control of cricket giant fibres

THE central nervous system not only receives sensory input, but also controls the nature of that input. Efferent control of sensory pathways was suggested on the basis of behavioural experiments by von Holst and Mittelstaedt¹ and by Sperry², who called the postulated mechanism efference copy and corollary discharge, respectively. They argued the need for an efferent system to compensate for the stimulation received during behaviour, and which would otherwise be interpreted as due to events occurring in the environment.

An early example of efferent control was the gamma system of vertebrate muscle spindles³, and since then several examples of control of sensory pathways at various points, from the primary receptors to deep within the central nervous system, have been described^{4,5}. But it has been difficult to show that efferent pathways carry efference copy or corollary discharge. We have now shown, using three experimental techniques, that two second order neurones in the house cricket, *Acheta domestica*—the lateral and medial giant interneurons (LGI and MGI)⁶⁻⁹, the largest axons in the abdominal connectives—are under efferent control. We have also shown that the control blocks impulses in these neurones, and that the efferent inhibition is operative in the behavioural circumstances that would result in self-stimulation.

Recordings were obtained from free-moving animals by securing a pair of 50 μ m wires around one connective, anterior to the terminal abdominal ganglion¹⁰. Each animal was tethered by the recording leads, in a 12 \times 12 inch arena with sand over the floor. The stimulus was a continuous 12 Hz sine wave contaminated by distortion in the output of the speaker.

Good control of stimulus parameters and clear identification of the LGI and MGI were achieved in restrained but minimally dissected crickets. After removal of the wings and the leg at the coxotrochanteral joint, each cricket was mounted ventral side up, and a flap of abdominal cuticle was

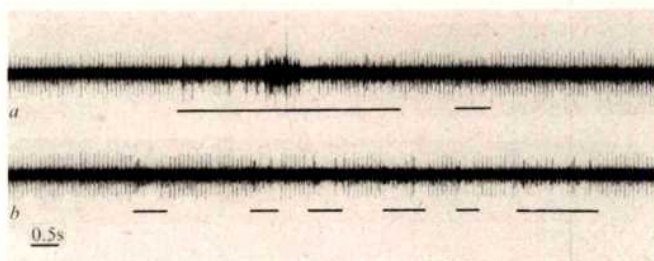


FIG. 1 Inhibition of giant fibres during running and grooming in a free-moving cricket with implanted electrodes (periods of running (a) and grooming (b) behaviour are marked with black bars). The animal was stimulated with 12 Hz tone which elicited giant fibre activity at each cycle; this activity was suppressed during episodes of behaviour.

removed. Silver wire electrodes surrounded by petroleum jelly recorded electrical activity in the two connectives independently.

Intracellular recordings were obtained from the dendrites of the MGI, as described elsewhere¹¹. Briefly, the animal was dissected from the dorsal side and the terminal ganglion was supported by a spoon-shaped holder; suction electrodes were attached to one abdominal connective and to the ipsilateral main leg nerve of the metathorax. A micropipette filled with Procion yellow¹² or potassium acetate was inserted through the sheath, and the neuropile was probed for the large dendrites of the MGI, which were identified by their responses to sensory stimulation and their electrical characteristics^{6,7,11}.

In free-moving preparations large spikes occurred with every cycle of the low frequency sound stimulus. During each bout of running the large units were silenced, although the stimulus continued unchanged (Fig. 1a). The inhibition was also correlated with other movements of the metathoracic legs, such as grooming (Fig. 1b). The experimenter's taped verbal account of the animal's behaviour is not a precise measure of the timing and specificity of the effect, but spike activity was monitored through an audio channel throughout each experiment and the results were unequivocal.

In restrained, minimally dissected preparations the LGI and MGI are reliably identifiable in the extracellular record. A touch to the head or thorax elicited running, that is vigorous waving of the leg stumps. In a quiescent animal, a tone pulse of 600 Hz and moderate intensity elicited a burst of spikes. The response was inhibited profoundly during running episodes (Fig. 2). On average, the response to strong stimuli (90 dB) was diminished less than the response to weaker stimuli (80 dB), but within this range of intensities the units were sometimes silenced completely.

In the restrained and highly dissected preparations used for intracellular recording, the MGI received inhibitory post-synaptic potentials (i.p.s.p.s.) during spontaneous bursts of spikes recorded from the ipsilateral metathoracic main leg nerve. During spontaneous motor activity, recordings from the dendrites of the MGI showed a hyperpolarisation of as much as 8 mV, which was maintained throughout the motor activity and sometimes extended beyond it (Fig. 3b). The MGI was often inhibited before the first observable motor spike. It has been suggested that efferent control of the lateral giant interneurone of crayfish is mediated by presynaptic inhibition¹². Many of our results might have been interpreted in a similar way since there was often a considerable decrease in excitatory end plate potential (e.p.s.p.) amplitude without obvious i.p.s.p.s. In other cases, however, i.p.s.p.s. were clearly recorded in the MGI dendrite, thus excluding a purely presynaptic mechanism. We could not demonstrate any direct correlation between unitary i.p.s.p.s. and motor nerve spikes. We conclude that the linkage between motor neurone activity and MGI inhibition is indirect, possibly provided by

an inhibitory neurone which receives excitatory input in common with the motor neurones.

The source of the descending inhibition was localised by selective cutting of interganglionic connectives. Inhibition was blocked by bilateral connective section anywhere between the third thoracic and terminal abdominal ganglia, but not anterior to the metathoracic ganglion. Thus the metathoracic ganglion is sufficient to produce inhibition. The possibility of additional inhibitory influences descending from more rostral centres remains open.

Most inhibitory influence on the LGI and MGI was abolished by sectioning of the connective ipsilateral to the axon of interest, while cutting the contralateral connective had a small effect. In contrast, the smaller ascending giant interneurons which fire tonically receive their inhibitory control equally from the two connectives. This pattern of connections correlates well with the pattern of sensory connections of the same neurones and with their dendritic field shapes: the LGI and MGI receive virtually exclusively ipsilateral excitatory input from the cerci and have unilaterally distributed dendrites, while the smaller, tonically active units receive bilateral input and have bilaterally distributed dendrites⁷.

Another inhibitory effect, which was completely independent of the thoracic centres, was also observed. When stimulated to run by a puff of wind aimed at the body, a reduction in the response to a standard tone could be detected even after severing all connections between the terminal ganglion and the remainder of the nervous system. This reduction in response might be due to lingering air movements or inhibitory circuits within the terminal ganglion.

Although the exact function of the giant fibres in controlling evasive behaviour has not been determined⁹, powerful stimulation of cercal receptors, which excites the giant fibres

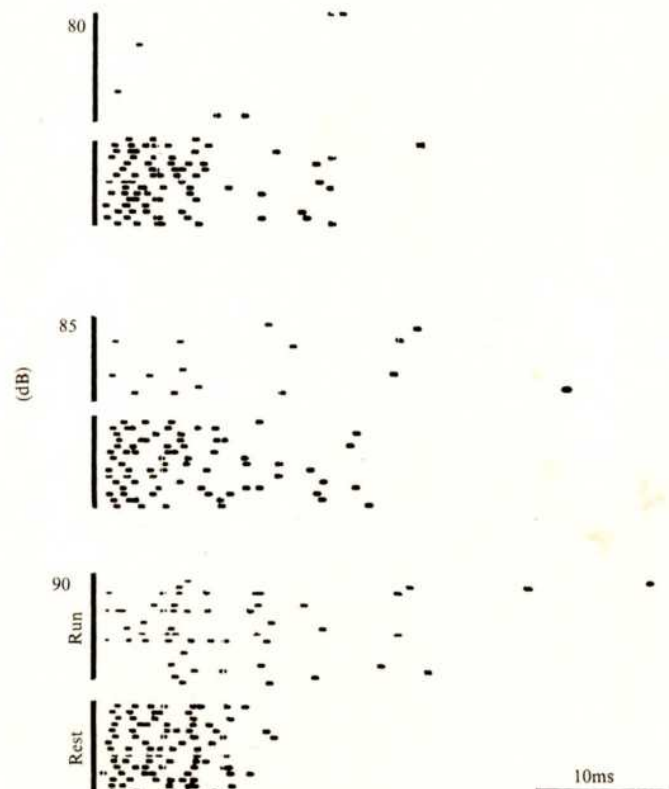


FIG. 2 Response of the two largest interneurons (LGI and MGI) to tones during periods of motor activity and rest. Each dot indicates the occurrence of a single spike, each row a single trial. For each stimulus intensity, indicated in the upper left hand corner, fifteen repetitions during touch-elicited running (above) and fifteen repetitions during rest (below) are shown.

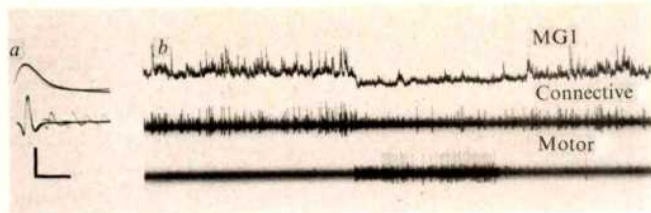


FIG. 3 Inhibition of the MGI during motor activity. (a) demonstrates that spikes recorded from the impaled neuron (top trace) are correlated 1:1 with spikes recorded extracellularly from the connective anterior to the terminal ganglion (lower trace). (b) shows that a spontaneous burst of motor activity (bottom trace) is correlated with a hyperpolarisation of the MGI (top trace) and a decrease in tonic activity in other giant fibres recorded extracellularly (middle trace). Calibrations: vertical *a*, upper, 40 mV; *b*, upper, 8 mV; horizontal *a*, 12 ms; *b*, 200 ms.

as a group, often elicits escape jumps. Therefore, excitation of the cercal receptors such as may occur during grooming or locomotion, might elicit evasive behaviour which would be inappropriate because the stimulus resulted from the animal's own behaviour and not from environmental events. On the other hand, escape is a high priority behaviour, and is not likely to be blocked completely during grooming or casual locomotion. In fact, the efferent inhibition can be overcome by strong stimulation (Fig. 2). Thus it seems that these neurones' thresholds are modulated to preserve the significance of their spike activity during behaviour. This could be interpreted as a simple example of the functioning of efference copy or corollary discharge.

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Chloride-related Depolarisation of Crayfish Muscle Membrane Induced by L-Glutamate

THE action of L-glutamate is localised at the junctional region of the muscle membrane in Crustacea and mimics that of the neurotransmitter in various characteristics. It is therefore possible that L-glutamate is the excitatory transmitter of the crustacean neuromuscular junction^{1,2}.

The excitatory junctional potential (e.j.p.) and glutamate potential induced by iontophoretic application of L-glutamate require Na but not Cl (refs 3, 4), but Cl was essential for depolarisation induced by the drug in the present experimental conditions.

The experiments were carried out on the opener muscle of an isolated walking leg of the crayfish (*Cambarus clarkii*). The experimental procedures, conditions and physiological solution were almost the same as those described elsewhere⁵. Propionate (Tokyo Kasei Kogyo, Co.) was used as the non-permeable and inert ion⁶⁻⁸ to replace all the Cl in the modified Van Harreveld's solution. To determine changes in resting potential, a coarse microelectrode filled with 3 M KCl was used as an indifferent electrode. Experiments were usually performed at room temperature (21°–22° C).

When L-glutamate (10⁻⁴ M) was added to normal medium, the muscle membrane was depolarised by about 10 mV after 1 min, and began to repolarise but stayed at a depolarised level a few minutes later, while the e.j.p.s. gradually disappeared at this time (Fig. 1*Aa*). On the other hand, when Cl was removed from the medium, the membrane was not depolarised but hyperpolarised by the drug (Fig. 1*Ab*). The large e.j.p. attained after the removal of Cl abruptly disappeared after the drug application, in contrast to the decreasing disappearance of the e.j.p. in normal medium (Fig. 1*Aa*).

This suggests that Cl is required for glutamate depolarisation in this case, provided that propionate replaced with Cl is inert to the postsynaptic membrane (Fig. 1*Aa,b*). The lack of glutamate response in Fig. 1*b*, however, may not be due to the accelerated desensitisation, because when glutamate was quickly added by iontophoretic application, the drug could

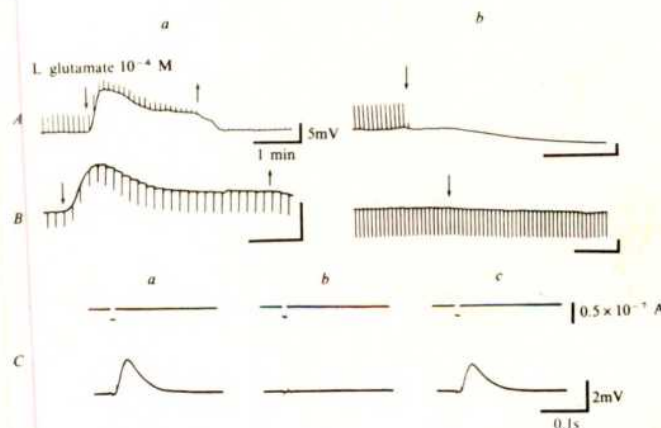


Fig. 1 Effect of chloride withdrawal on L-glutamate-induced depolarisation and input resistance. *A*: Excitatory axon was stimulated by a train of 8 impulses at 90 s⁻¹ every 6 s, and the e.j.p.s. were displayed with a pen-writing recorder. The drug was added at the downward arrow by bath application and removed at the upward arrow. Calibration bars given for *A* and *B* are 1 min and 5 mV in all cases. *a*, Normal medium; *b*, 15 min after Cl removal. *B*: Two microelectrodes, one for recording and the other for passing current, were inserted into the middle of a muscle fibre. Electrotonic potential was produced by passing a current pulse of 2×10^{-8} A across the muscle membrane. *C*: L-Glutamate was applied iontophoretically at a junctional region after Takeuchi and Takeuchi². *a*, In normal solution; *b*, during withdrawal of Cl; *c*, after returning to the normal solution.

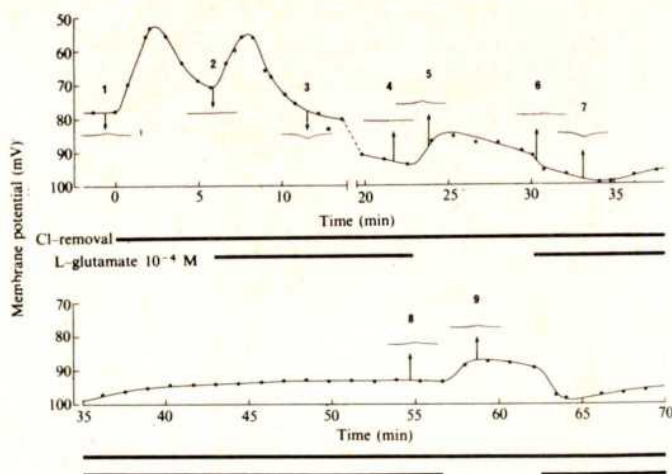


Fig. 2 Effects of L-glutamate on resting potential and inhibitory junctional potentials. The resting potential was monitored with a pen-writing recorder and the i.j.p.s that were induced by stimulating the inhibitory axon with a train of 8 impulses at 90 s^{-1} were displayed at each arrow by oscillographic means. Vertical bar at arrow 1 shows 1 mV for i.j.p., but 2 mV for a dotted i.j.p. The medium was perfused with a Cl-free solution at the upper bar and the drug was added at the lower bar by perfusing a preparation with a solution containing the drug. At arrows 7, 8 and 9, the same as arrows 3, 4 and 5 was repeatable, but 1 h after the Cl removal the hyperpolarising 'i.j.p.' was not observed further after drug application.

not produce any potential with Cl removal (Fig. 1C). This mode of drug action differed from that previously reported and that of the neurotransmitter²⁻⁴. There should therefore be another site of action for L-glutamate at which Cl is playing an essential role.

Effective resistance increased with depolarisation after the drug application (Fig. 1Ba). L-Glutamate, however, produced neither a depolarisation nor an increase in effective resistance after Cl removal (Fig. 1Bc). A simple interpretation of these facts is that Cl may carry all the depolarising current induced by L-glutamate without any considerable conductance increase, although this is rather complex because of the underlying non-linear relationship between the current and the voltage^{9,10}. Since the drug depolarised the membrane well beyond the Cl potential (Fig. 1Aa) that is usually found close to the resting potential¹¹⁻¹³, this amount of depolarisation cannot be brought about by an increase of Cl conductance alone, if at all. The Cl therefore must be 'pumped out' against an electrochemical gradient in this case. The experiments shown in Figs 2 and 3 may support this theory, although a possibility that Cl is indispensable for the depolarisation induced by other ions is not completely excluded.

With Cl removal the membrane potential was largely depolarised but began to repolarise after a few minutes (Fig. 2), while a depolarising i.j.p. induced by repetitive stimulation of the inhibitory axon (arrow 1) disappeared several minutes later (arrow 2). In this condition, L-glutamate still produced the depolarisation, presumably because some amount of internal Cl remained. About 2 min after drug application, the membrane began to repolarise and became hyperpolarised beyond the original level. During repolarisation, a large hyperpolarising 'i.j.p.' appeared in spite of a Cl-deficient condition as shown at arrow 3. The 'i.j.p.' became smaller in size with the hyperpolarisation of the membrane and disappeared at arrow 4. Since the i.j.p. is induced by selective conductance increase of Cl (refs 11, 14), this experimental result indicates that a concentration gradient of the ion across the membrane has become high at least at the synaptic region after the drug application. The greater amount of external Cl beyond that predicted from the Nernst equation should be pumped out and retained there for a while. A considerable amount of Cl can be expected to remain inside the muscle fibre even in Cl-free medium¹⁴⁻¹⁶.

When L-glutamate was removed from the medium, the i.j.p. began to go positive (arrow 5) and then diminished at arrow 6, suggesting that the amount of external Cl 'pumped out' decreased after withdrawal of the drug. Although the possibility that K or propionate may become permeable when the inhibitory synapses work under the glutamate action has not been excluded, this experimental fact can more reasonably be accounted for by the stimulating action of the drug for the 'Cl-pump' at the junctional region than by the interaction between the drugs.

When the temperature of a preparation was varied by perfusing it with cold or warm solution, glutamate potential altered greatly depending on the temperature (Fig. 3). With cooling, the potential became smaller and finally disappeared when cooled below 15°C in this preparation. The potential reappeared and was restored to original size by warming the muscle fibre. The peak time of these potentials showed little change in the course of this procedure^{6,17,18}. Essentially the same result was obtained in a case where the drug was applied by the perfusing procedure. The temperature at which the glutamate potential disappeared depended on the preparation and the quantity of the applied drug, ranging from 15° – 19°C .

From these experimental results it seems likely that the Cl-related depolarisation produced by L-glutamate was brought about by an 'electrogenic Cl pump', at least in part, which might be loosely coupled with the 'Na pump'. The slow hyperpolarisation after the drug application and the depolarisation after removing it in Fig. 2 suggest a hyperpolarising action of the drug in addition to the depolarising action. The depolarising action would be dominant in a normal medium. In this connection, it is interesting that this type of active transport has been found in various tissues of many kinds of animals¹⁹⁻²⁴. It is also likely that this type of activity may prove to be of importance in the control of the synaptic activities or the long-term reaction in the muscle^{25,26}.

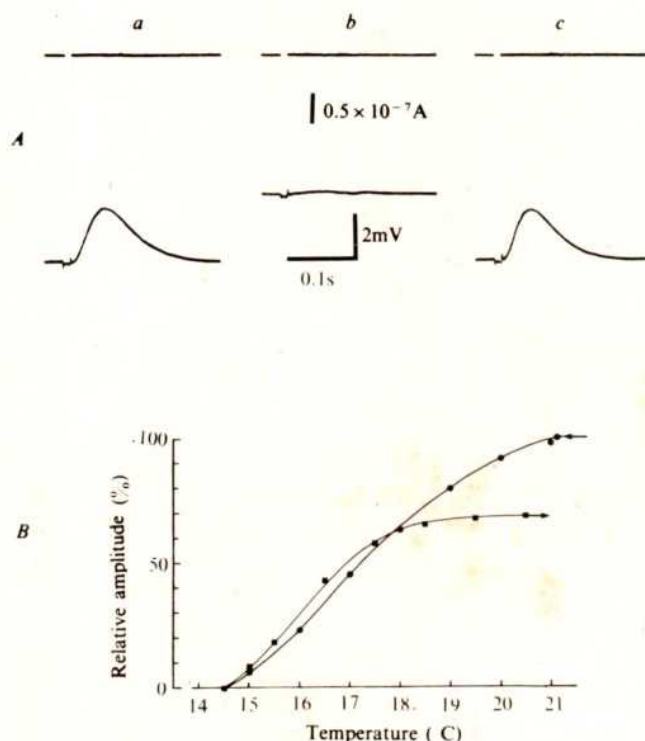


Fig. 3 Effect of temperature on glutamate potential. L-Glutamate was applied iontophoretically at a junctional region every 12 s. The preparation was perfused with cold or warm solution and its temperature was read on a thermometer inserted close to the preparation in the bath. A: a, at 21°C ; b, below 15°C ; c, after rewarming the preparation to 21°C . B: Relative amplitude of the glutamate potentials plotted against temperature. Arrows show experimental procedure.

Some dissociated characteristics between the e.j.p. and the glutamate potential should be subjected to further investigation so that L-glutamate may obtain a definite qualification as a candidate for the excitatory transmitter at the crustacean neuromuscular junction.

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Extracellular Currents from Frog Photoreceptors

EVIDENCE has been presented to show that dark current flows around vertebrate photoreceptors^{1,2}. The outer limb is a sink for this current; the remainder of the receptor is a source. The effect of light is to reduce the current by increasing the resistance of the outer limb membrane^{3,4}. Some authors have, however, suggested complications to this hypothesis^{5,6}. The problem was therefore reinvestigated for the frog, *Rana esculenta*.

Our methods were modelled on those described by Hagins *et al.*^{1,2}, but instead of retinal slices, whole retinas were mounted on a holder consisting of two perspex plates hinged by a collodion film. When this holder was folded, the receptor edge of the retina protruded through a 1 mm long slit in the collodion. The holder was placed between two pools of Ringer on a microscope slide, and the receptor edge could be viewed by infrared microscopy. The edge was moved onto an array of

microelectrodes and recordings were made of potential differences between electrode pairs. Changes in potential were observed when there was a change of electrode position relative to the retina, or when the retina was stimulated. Stimulation consisted of constant current pulses applied across the edge followed by light flashes perpendicularly incident to the receptor axes.

The records of Fig. 1 were obtained from two electrodes on a line at right angles to the edge. One electrode remained fixed, as the edge was advanced upon it; the other was always adjusted to a given reference position at the receptor tips. The bathing medium contained aspartate ions to simplify the potentials⁷. Little response to light was seen when the retinal electrode was 30 μ m deep (Fig. 1a). At 45 μ m it registered a small response which long outlasted the stimulus (Fig. 1b). This region of the retina contains mostly rod outer limbs. At 60 μ m and beyond (Fig. 1c-g) the electrode was among both rods and cones and recorded two components, the prolonged one previously seen and a fast one with a time course matching the stimulus. Individually the two components resemble intracellularly recorded rod and cone receptor potentials⁸.

The relationship between dark voltage and photovoltage was examined in the experiments of Fig. 2. The electrodes remained

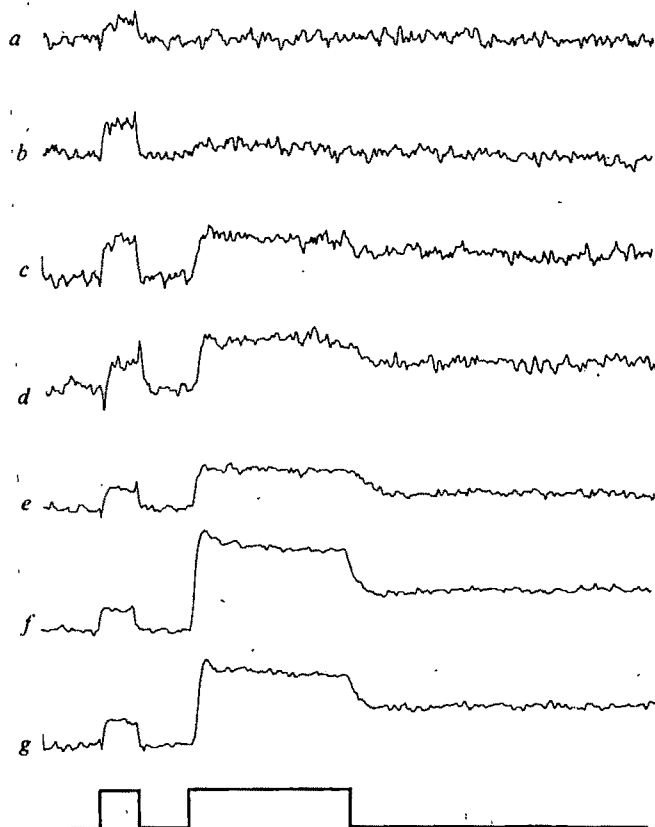


Fig. 1 Average voltages across receptor layer due to current pulses and responses to light flashes, repeated two to four times at each depth. a, 0–30 μ m; b, 0–45 μ m; c, 0–60 μ m; d, 0–75 μ m; e, 0–90 μ m; f, 0–105 μ m; g, 0–120 μ m. 0 μ m corresponds to the receptor tips. Calibration deflections mark the occurrence of current pulses (duration: 240 ms), and light flashes (1 s). The amplitude of the deflections corresponds to 15.6 μ V (a–c), 31.3 μ V (d), and 62.5 μ V (e–g). Current pulses were 5.5 μ A (a–e) and 1.7 μ A (f–g). Upward deflection recorded when current flowed receptor to ganglion side. Light flashes: 561 nm, 3.7×10^{13} $h\nu$ cm^{-2} s^{-1} , incident perpendicularly to the receptor axes. This light intensity produced a nearly saturated cone response but had no observable effect on the rod response when repeated at intervals of more than 40 s. Bandpass: 0–16 Hz. Electrode tips: about 2 μ m, 8 M Ω , filled with Ringer. Ringer: NaCl 72, KCl 1.8, CaCl₂ 0.1, MgSO₄ 0.1, NaH₂PO₄ 2.5, Na₂HPO₄ 10, glucose 10, monosodium aspartate 10 mmol l^{-1} (pH=7.2–7.3).

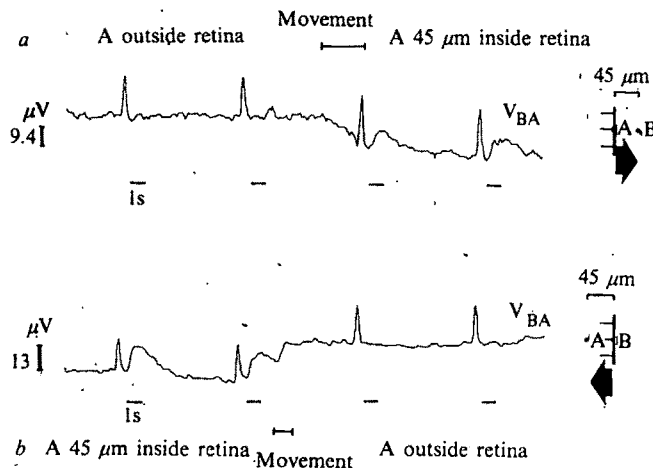


Fig. 2 Continuous records showing responses to light flashes and DC changes accompanying changes in the position of the electrodes relative to the retina. Position of electrode tips, B and A, before movement and direction of movement of the retinal edge is indicated to the right of each record. Current pulses flowing receptor to ganglion side precede the light flashes and produce voltage 'spikes'. Downward deflection corresponds to positivity of A. Light flashes: 1 s, 561 nm, 3.7×10^{10} $\text{h}\nu \text{ cm}^{-2} \text{ s}^{-1}$. Horizontal lines mark the duration of the flashes and of the movements. The light intensity saturated the rod response without eliciting any noticeable cone activity. Bandpass: 0–1.6 Hz. This severe filtering lengthens the rise time of the rod response but has been shown not to affect its maximum amplitude. Electrode tips: 1–3 μm , 6–16 $\text{m}\Omega$, filled with Ringer. *a*, A changes position, 0–45 μm from receptor tips. Ringer: see Fig. 1 except 82 mmol l^{-1} NaCl and no aspartate. *b*, A changes position 45–0 μm . Ringer: see Fig. 1 except 10 mmol l^{-1} monosodium glutamate instead of aspartate.

stationary throughout and the edge was moved so that one electrode (A) entered the retina (Fig. 2*a*) or came out of it (Fig. 2*b*) while the other (B) was always outside. For currents originating in the retina, it was as if B always remained at the receptor tips, since within experimental error the region outside the retina was isopotential with the edge. The results shown are typical of more than seventy experiments, some performed with standard Ringer (such as Fig. 2*a*), some with aspartate or glutamate Ringer (such as Fig. 2*b*). They represent the potential recorded by B minus the potential recorded by A (V_{BA}). Entry of the electrode into the outermost 45 μm of the retina always produced a downward shift in the DC level (A goes positive relative to B) followed by the appearance of upward-going photoresponses, the amplitudes of which were never greater than the shift. When the electrode came out, there was an upward DC shift and the responses disappeared.

Two types of effect were seen when movements of the edge brought the retinal electrode to the region of the outer limiting membrane (about 90 μm). In about one third of the cases the retinal electrode recorded increasing positivity (usually between 50 and 100 μV), as would be expected if a dark current flowed from the inner regions of the receptors to their outer limbs. But in most cases, during the last stages of the movement the increase in positivity was interrupted by a negative shift of the DC level (a millivolt or more), but most of the shift proved transient. When the retina was moved off the electrodes, no big shifts were seen. Then about two thirds of the records showed a decrease in positivity of A matching the diminution of the photoresponses. Probably the big DC shifts arise when an electrode tip disrupts the tightly packed structure of the inner receptor layer⁹.

In a few experiments giant, unstable photoresponses, up to a millivolt, were recorded from electrodes reaching the region of the outer limiting membrane. But the voltages produced by current pulses flowing parallel to the receptor axes (in the so-called 'radial' direction) remained the size expected for the

electrode position, suggesting that the major part of the large photoresponses was not generated across the radial extracellular resistance. Similar giant responses have previously been obtained at various depths in the frog receptor layer¹⁰. They probably arise in much the same way as the large extracellular responses that can be recorded when an electrode contacts a single spinal motoneurone¹¹. In any case giant responses, like the transient DC shifts, mask the relationship between radial dark voltages and photovoltages, and both types of effect have been excluded from subsequent analysis.

Measurements of the potential difference between two points along the extracellular path parallel to the receptors, as in the experiments of Fig. 2, do not allow components from local sources and sinks to be distinguished from those of remoter origin. But if potential measurements are made at three successive points (C, B and A) in a radial direction, then given certain assumptions, the potential differences, V_{BA} and V_{CB} , can be used to compute I , the current arising or disappearing between C and A^{2,12}.

$$I = (i/v_{BA}) \{ V_{BA} - (v_{BA}/v_{CB}) \cdot V_{CB} \} \quad (1)$$

where v_{BA} and v_{CB} are the voltages generated by constant current pulses of magnitude i applied to flow along CA.

Equation (1) was used to determine photocurrents and dark currents from the photovoltages shown in Fig. 1 and from the corresponding dark voltage changes (not shown) produced by changes in electrode position. The results can be seen in Fig. 3.

Consider first the leading edge of the photocurrent. About 680 current units were generated in the layer 30–60 μm deep, while about 370 disappeared in the layer 60–90 μm , and 420 in the layer 90–120 μm . If about 110 units were produced in the layer 0–30 μm but could not be detected owing to the

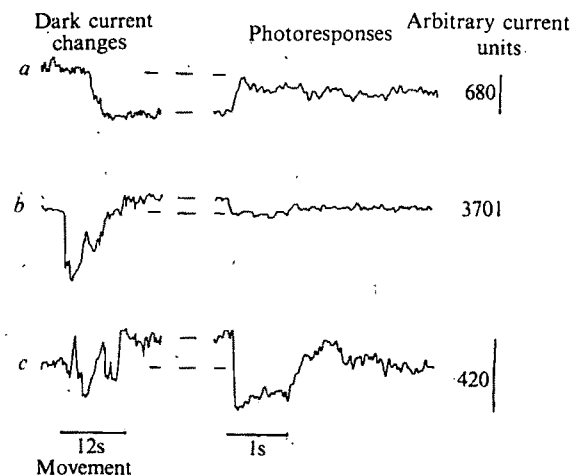


Fig. 3 Current sources and sinks 30–120 μm from receptor tips. Experimental details: see legend to Fig. 1. The dark voltage changes for each 15 μm change of electrode position were measured directly. The voltages across each 15 μm layer due to current pulses and photoresponses were obtained by subtracting the successive records of Fig. 1 from each other. The potential differences across two adjacent 15 μm layers were scaled to compensate for resistance differences between the layers, subtracted from each other and converted to currents according to equation (1). Currents at three depths in the receptor layer are shown: *a*, outer limb region, 30–60 μm ; *b*, inner limb and myoid, 60–90 μm ; *c*, nucleus and synaptic region, 90–120 μm . For a given depth the dark current changes are compared with the photoresponses plotted on the same scale. The scale is indicated in arbitrary current units to the right of the photoresponses. Arbitrary current units are used because of problems in accurately defining the geometry of the retinal edge. The area of the exposed edge was of the order of 1 mm^2 . The dotted lines indicate the dark current before and after the retinal movement with its transient disturbances. Upward shifts represent current sources, downward shifts sinks. Bandpass for dark current changes: 0–1.6 Hz; for photoresponses, 0–4 Hz.

low resistance of the layer, all the photocurrent is accounted for and this must have arisen from the receptors, since up to about 90 μm no other cells are present. Likewise the dark current up to 90 μm can only have originated in the receptors. Its magnitude is in agreement with that of the photocurrent but is opposite in sign. Such a result provides evidence for the view that receptor photocurrents represent reductions of dark current, at least in the aspartate-treated retina.

For the layer 90–120 μm , there is a discrepancy between the size of the dark current actually observed and that needed from the layer to balance the sinks in the outer limbs. This implies that the layer contains not only receptor sources of dark current but also non-receptor sinks balanced by sources still deeper in the retina. Non-receptor dark currents account for a further discrepancy in Fig. 3c. Thus the net dark current source (receptor source + non-receptor sink) is smaller than the leading edge of the photocurrent (receptor alone). In other experiments, sinks of dark current in the inner receptor layer were even more pronounced, especially if no aspartate was added to the Ringer solution.

Figure 3 shows that, in addition to non-receptor dark currents in the innermost receptor layer, there are non-receptor photocurrents. The response waveforms of Fig. 3a and b match each other but are different from that of Fig. 3c. The result is consistent with the idea that there is a source of photocurrent in the layer of Fig. 3c, which develops slowly and reaches its peak after the cone and rod sinks have passed theirs. Possibly the source is in the outer ends of the Müller cells and gives rise to what has been called slow PIII in other vertebrates^{13,14}.

The existence of variable non-receptor sinks and sources of current in the receptor layer could perhaps explain some of the differences between the results of various studies. Further analyses of receptor function based on extracellular current measurements, especially those of the innermost receptor layer, will have to take non-receptor currents into account.

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Nematode growth factor

FREE-living nematodes, grown axenically in a chemically defined medium, might be of considerable interest as model systems for studying the fundamental aspects of genetics and differentiation of higher organisms.

Several species of free-living nematodes have been cultured serially in axenic media¹. Unfortunately, the chemically defined medium in current use, *Caenorhabditis briggsae* Maintenance Medium (CbMM)², does not support continuous growth unless supplemented with a 'growth factor' that can be derived from various biological sources. Conventional growth factor, thus prepared is proteinaceous and biologically highly active. When added as a supplement to the chemically defined medium it supports growth and maturation of the commonly used test organism *Caenorhabditis briggsae* within 3.5 (yeast ribosomes) to 5 d (heated liver extract) and continuous development for several generations, resulting in large final populations. The biological activity is associated with precipitate formation²⁻⁴.

It has been shown that *C. briggsae* has a nutritional requirement for sterols and haem^{5,6} that must be provided by the growth factor as they are not included in the basal medium. The proteinaceous portion of the growth factor has been substituted with some success by several commercially available proteins⁷. Substitution of the protein portion by heat stable material, such as glycogen, is also effective and as with growth factor, the biological activity is associated with the precipitated portion of the medium⁸.

Thus the current idea is that the growth factor consists of sterols, a haem moiety and organic material which might provide nutrient in the proper particulate form⁸.

I now wish to further clarify the nature of the growth factor for *C. briggsae*. My hypothesis is that growth factor contributes only two dietary essentials (sterols and haem) to the basal medium and that the third portion of the growth factor, whatever its chemical nature might be, serves only as a convenient vector of the haem component, which must be supplied in a particulate form. This implies that any material should be able to substitute for the third component of the growth factor, provided that it is not toxic to the nematodes, capable of 'carrying' an iron protoporphyrin complex, either by containing haem (such as a haem protein) or by binding an effective substitute (haemin chloride) and available to the nematodes in particulate form.

I have confirmed the growth promoting activity of ribosomes from yeast^{3,4} and *E. coli*⁵ and obtained the same activity with ribonucleoprotein (RNP) particles from bovine liver and HeLa cells. Pea seedling-RNP particles were unable to support growth but their activity was restored upon addition of haemin chloride. This supports the theory as most RNP particles washed only in low salt buffers, are easily contaminated with a haem protein (most probably a cytochrome⁹). *E. coli* ribosomes washed with 1 M salt are inactive, as could be expected.

In another set of experiments, several purified haem proteins were shown to be highly active when properly precipitated and added to the basal medium, containing sterols.

In a third series of experiments, iron binding proteins were allowed to interact with haemin chloride and then carefully precipitated and tested for their growth promoting activity. Some of these aggregates (especially ferritin-haemin and transferrin-haemin) proved to be as active as growth factors, derived from natural sources. Less specific iron-binding material as bovine serum albumin, albumen or conalbumin permits only limited development of *C. briggsae* with a longer generation time.

In the next set of experiments, the 'carrier' portion of the growth factor was substituted by the phospholipid lecithin. Egg lecithin was dispersed ultrasonically and haemin chloride

was allowed to interact with the phospholipid particles before addition to the basal medium containing sterols. It was expected that lecithin-haemin complexes might be formed (both are lipophilic), providing haemin in particulate form. Lecithin, supplied at a final concentration of 10 mg ml⁻¹ proved to be toxic. At concentrations of lecithin 1 mg ml⁻¹, haemin 50 µg ml⁻¹, *C. briggsae* developed continuously for several generations (Table 1).

Details of the above experiments will be given elsewhere. Although the results, reported here, do not allow definitive conclusions about the minimal chemical composition of the growth factor, they strongly support the hypothesis that the third component of growth factors, derived from natural sources, is primarily a matter of function (as a convenient

vector of haem) rather than of chemical structure. This suggests a trivial explanation for the failure of previous trials to elucidate the nature of the growth factor by means of sophisticated biochemical purification.

Conclusive proof that the iron protoporphyrin constituent in any particulate form is the active component in sterol supplemented medium, is given by the fact that a medium, composed of CbMM, sterols and precipitated haemin only, will support continuous growth and reproduction of *C. briggsae*. The experimental design was based on the apparent irreversibility of haemin precipitation in acid medium. When the haemin suspension is brought to pH 5-5.4, haemin does not redissolve appreciably. This pH range is still compatible with the acid pH tolerance of *C. briggsae*. The results of two experimental arrangements are represented in Table 2. In these experiments, growth, comparable with that supported by natural growth factors, has been obtained with concentrations of 25 to 100 µg haemin ml⁻¹. In general, there are only minor differences between the first and the second experiment, probably due to different pH and salt concentration (high concentration of salt and low pH are both unfavourable). Higher concentrations of haemin (200 µg ml⁻¹) inhibit fast growth in both experiments. Although the influence of salt cannot be excluded, indications from former experiments suggest that haemin itself becomes toxic at higher concentrations.

Up till now *C. briggsae* has been maintained axenically through 10 serial subcultures in this medium (haemin at 100 µg ml⁻¹; pH = 5.0) without change in growth rate. CbMM supplemented with sterols (50 µg ml), haemin chloride (50 µg ml⁻¹) and 3% v/v acetic acid (pH of final medium 3.5) supports also growth and maturation of the

TABLE 1 Biological activity of lecithin-haemin particles

Final concentration of supplement	Generation time (d)	Increase over inoculum after 28 d
Lecithin 10 mg ml ⁻¹		
Haemin 10 µg ml ⁻¹	nm*	—
Lecithin 10 mg ml ⁻¹		
Haemin 50 µg ml ⁻¹	nm*	—
Lecithin 1 mg ml ⁻¹		
Haemin 10 µg ml ⁻¹	7-10	× 2†
Lecithin 1 mg ml ⁻¹		
Haemin 50 µg ml ⁻¹	6-9	× 250‡

Experiment carried out in four replicates. Sterols were included at a final concentration of 50 µg ml⁻¹ (ref. 5).

* nm indicates non-maturing.

† Only a few larvae emerged but failed to develop.

‡ Thriving cultures; F₂ larvae produced.

TABLE 2 Growth of *C. briggsae* on chemically defined medium containing sterols and supplemented with various concentrations of precipitated haemin

Haemin µg ml ⁻¹	F ₁ generation time (d)	F ₂ generation time (d)	Population at 7 d	Population at 14 d	Population at 21 d	Egg mass at 21 d
First precipitation method. Final pH of complete medium = 5.0*						
	(16)			(8)	(8)	(8)
200	7.5 ± 0.15	uncertain	nd	20 ± 4	376 ± 95	absent
100	6.2 ± 0.1	12 to 14	nd	75 ± 11	1,127 ± 103	large
50	6.0 ± 0.1	12 to 14	nd	146 ± 17	1,583 ± 232	large
25	6.0 ± 0.1	12 to 14	nd	126 ± 19	1,140 ± 94	small
12.5	6.1 ± 0.1	13 to 14	nd	96 ± 22	567 ± 50	very small
6.25	6.0 ± 0.1	uncertain	nd	32 ± 5	154 ± 25	absent
3.1	6.0 ± 0.12	no F ₂	nd	24 ± 6	59 ± 9	absent
1.6	6.2 to nm	no F ₂	nd	8 ± 3	29 ± 6	absent
Second precipitation method. Final pH of complete medium = 5.4†						
	(20)		(8)	(8)	(4)	(4)
200	6.9 ± 0.4	uncertain	4 ± 1	25 ± 5	172 ± 97	absent
100	5.6 ± 0.15	11 to 13	21 ± 6	185 ± 46	2,109 ± 145	very large
50	5.2 ± 0.15	10 to 11	26 ± 3	790 ± 62	1,401 ± 358	very large
25	5.0 ± 0.1	10 to 12	36 ± 6	598 ± 53	662 ± 75	small
12.5	5.3 ± 0.2	no F ₂	21 ± 3	50 ± 7	67 ± 13	absent
6.25	7.5 ± 0.6	no F ₂	8 ± 1	16 ± 5	28 ± 7	absent
3.1	7.5 to nm	haemin dissolves again: no further growth supported				
1.6	nm					

Mean values ± s.e. are given where possible. The number of replicates is given in brackets. Replicates were grouped in sets of four. Culture tubes containing 0.25 ml of medium were inoculated with three newly hatched larvae and incubated at 21-22°C. F₁ generation time is measured as the time from the incubation of these larvae to the production of larvae of the next generation. F₂ generation time is defined as the time required for two consecutive reproductive cycles. Media are evaluated further by the size of the population originating from the three initial larvae (counts of the cultures *in toto*). The size of the egg masses produced eventually after 21 d of growth provides an additional criterion. nd, Not determined; nm, not maturing.

* CbMM, containing sterols (50 µg ml⁻¹) was acidified with 1 N HCl to pH = 3.5 and subsequently supplemented with haemin chloride from a stock solution to obtain a final concentration of 400 µg ml⁻¹; in these conditions haemin precipitated immediately. This medium was then mixed with equal amounts of CbMM containing sterols (50 µg ml⁻¹) pH 7.67, thus giving a complete medium composed of CbMM, haemin at 200 µg ml⁻¹ and sterols at 50 µg ml⁻¹ with final pH of 5.0. This medium was then diluted serially with CbMM, pH 5.0, containing sterols (50 µg ml⁻¹) to obtain the concentrations of haemin indicated. This precipitation method implies the inclusion of high salt concentration in the medium.

† Haemin chloride was pipetted from stock solution into Hcl 0.01 N (pH = 2.0) to give a final concentration of 400 µg ml⁻¹. The suspension thus obtained, was then mixed with equal amounts of CbMM at twice the concentration, pH 5.9, containing sterols (100 µg ml⁻¹). The resulting complete medium is then composed of CbMM at 1× concentration, haemin chloride at 200 µg ml⁻¹ and sterols at 50 µg ml⁻¹ with final pH = 5.4. Serial dilutions were made from this medium with CbMM (containing sterols at 50 µg ml⁻¹) pH 5.4.

free-living nematode *Turbatrix aceti*. This organism has now already been maintained under aseptic conditions throughout nine serial subcultures.

Four main conclusions can be drawn from these findings. First, the growth factor for *C. briggsae* has been elucidated. This growth factor, it should be emphasised contributes only two dietary essential components to the basal medium: sterols and haem. Second, as the haem constituent can be substituted effectively by precipitated haemin chloride, a chemically fully defined medium for the axenic cultivation of *C. briggsae* is now available. Third, the fact that the haem component must be provided in particulate form to be efficient indicates strongly that it is taken up by phagocytosis. This hypothesis is now being checked. Fourth, it is quite conceivable and even probable that the elucidation of the growth factor for *C. briggsae*, may have significance far beyond the study of this single species, as suggested by the fact that supplements promoting growth of *C. briggsae* have also a stimulatory effect on growth of many other nematode species. In this respect, the successful axenic cultivation of *T. aceti* on a chemically fully defined medium may be highly significant.

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Depression of Morphine-seeking Behaviour by Dopamine Inhibition

MANY of the direct effects of morphine have been attributed to serotonergic mechanisms¹ or to the catecholamines^{2,3}. The role of brain monoamines in morphine's capacity as a behavioural reinforcer has only recently been explored^{4,5}, and it has been found that the catecholamine depletor α -methyl-*p*-tyrosine (AMT) blocks behavioural reinforcement by morphine. Our own work, started before these reports appeared, strongly suggests that dopamine is implicated in this blockade. To study morphine reward we adopted the technique of Beach⁶ which uses the acquisition and retention of a goal box preference and thereby minimises non-specific effects by experimental drugs on performance.

Sprague-Dawley male rats (180–300 g) were tested for pre-training preferences in a Y-maze with two contrasting boxes. Morphine dependence was induced by gradually increasing doses from 5 to 20 mg kg⁻¹ intraperitoneally twice daily, during 7 d. Training started with a saline injection in their home cages and an immediate forced run in the Y-maze to their preferred box where they were confined for 1 h. This was followed 20 min later by injection of 20 mg kg⁻¹ morphine and a forced-run to their non-preferred box and 1 h confinement therein. Training by forced runs was achieved by blocking off the opposite alley. We assume the rats learned to associate the effects of the injection with the response of running to the respective box. Sixteen free-choice trials (all barriers open) were presented on training days 15 and 16 (acquisition). Three weeks later, during which no treatment was administered (except see below), the rats were again tested with sixteen free choice trials (that is, 'relapse')⁶.

Our results show that the rats acquired and maintained a preference for their formerly non-preferred, morphine-rewarded box (group C-Mor, Fig. 1), in full agreement with Beach⁶. Choices of the latter were significantly greater than those of a group receiving saline alone (group C-Sal; $P < 0.01$), or compared with their own pretraining preference scores. No difference was found between these morphine-trained rats and parallel control groups receiving the carriers for the various modifying agents used below (Kruskal-Wallis analysis of variance⁷; d.f.=6; $P > 0.90$). These groups were therefore combined and are represented in Fig. 1 as C-Mor. All other P values were derived by the Mann-Whitney U test, two tailed⁷. That the learned preference was not an expression of avoidance of the 'withdrawal' box was shown by another control group run only to the preferred box. These rats selected the latter in 52 and 69% of their trials during the acquisition and relapse tests respectively, thus exhibiting no avoidance of this box. We, therefore, assume that the learned preferences are mediated by the euphoric and (or) the abstinence relieving components of morphine reinforcement^{8,9} rather than by avoidance of the opposite box, in line with the findings by Kumar¹⁰.

AMT, which has been repeatedly shown to deplete both noradrenaline and dopamine^{11–13}, was administered to another group of rats (80 mg kg⁻¹ intraperitoneally every 12 h) during morphine training. This treatment significantly depressed acquisition of a morphine-box preference ($P < 0.05$) and completely blocked relapse (Fig. 1). Initial choices of the morphine box were still greater than the C-Sal control group, indicating that the AMT only partially blocked morphine reinforcement. Catecholamine depletion by 6-hydroxy-dopamine (2 × 250 μ g intraventricularly, 2–4 d before training^{14–16}) in another group of twenty-one rats also significantly reduced acquisition and relapse similar to the AMT. Preferential depletion of serotonin by *p*-chlorophenylalanine¹⁷ (300 mg kg⁻¹ d⁻¹ for 3 d starting 5 d before morphine training and continued with 150 mg kg⁻¹ every other day during training) had no significant effect on preference scores in either test condition (Fig. 1).

Since AMT depletes both noradrenaline and dopamine, we tested to see whether one of these monoamines was more essential in mediating morphine reinforcement. Another group received the dopamine- β -hydroxylase inhibitor diethyldithiocarbamate (DETC), which depletes noradrenaline while dopamine levels remain unaffected or even increase¹⁸. DETC (400 mg kg⁻¹ injected daily 1.5 h before the morphine-reinforced trial) had no effect on morphine-box preferences (Fig. 1).

These data suggested that noradrenaline depletion was not critical in inhibiting morphine reinforcement. The role of dopamine was assessed in further groups treated with haloperidol which presumably blocks dopamine receptors¹⁹. Doses of 0.25, 0.5 and 1 mg kg⁻¹ haloperidol were administered 20 min before the daily morphine trials in separate groups. The 1 mg injections completely blocked morphine

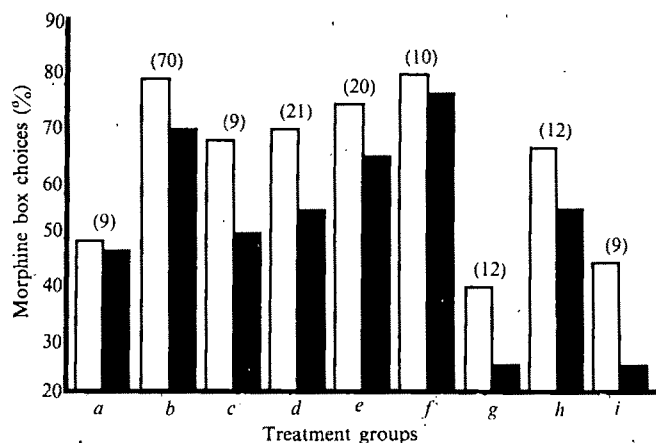


Fig. 1 Proportion of morphine-box choices (or non-preferred box as defined by pre-training preference trials) during free-choice tests after indicated treatments. Number of rats in each group shown in parentheses. Open columns, acquisition; black columns, relapse. a, C-Sal; b, C-Mor; c, AMT; d, 6-hydroxydopamine; e, *p*-chlorophenylalanine; f, DETC; g, 1 mg kg⁻¹ haloperidol; h, 0.5 mg kg⁻¹ haloperidol; i, AMT plus 0.5 mg kg⁻¹ haloperidol.

reinforcement whereas 0.25 and 0.5 mg produced a significant reduction compared with group C-Mor ($P < 0.05$ in each case). Combining the treatment schedules for AMT and 0.5 mg haloperidol in an additional group, however, significantly reduced morphine-box choices below that of either treatment alone ($P < 0.02$ in each case) and did not differ from the 1 mg haloperidol group (Fig. 1).

The possibility that the AMT interfered with original learning or later memory of the morphine-seeking habit was examined in twelve rats trained with morphine while treated with AMT exactly as before, but were re-addicted to morphine 1 week before relapse testing. These animals showed a mild decrease in initial choice tests, but during relapse testing they selected the morphine-rewarded box 70% of the time, indicating that AMT did not impair learning or memory as compared with the original AMT group. Neither did administration of the modifying drugs alone have any effect on preference scores, as shown by two groups receiving AMT or haloperidol but no morphine ($N = 12$ and 11 respectively). The selection of the non-preferred box by the AMT group was 42% and 35% for the haloperidol group, and did not significantly differ from the scores of group C-Sal.

The data indicate that interference with dopamine activity, in contrast to noradrenaline and serotonin, effectively reduces the behaviourally reinforcing effects of morphine. Dopamine reward mechanisms recently implicated in intracranial self-stimulation²⁰ may be involved; however, since AMT reduces certain abstinence symptoms in the rat²¹ and also makes morphine aversive²², the possibility is raised that the reward value of morphine was diminished by these effects as well.

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Appearance of Unusual Mitochondria in Rice Coleoptiles at Conditions of Secondary Anoxia

IN rice coleoptile cells mitochondria can be formed and preserved without any notable signs of destruction in the absence of molecular oxygen¹⁻³. In this, rice coleoptiles are strikingly different from other plant tissues, whose fine cell organisation is degraded during even a short-term anaerobiosis⁴⁻⁶. In the experiments mentioned, however¹⁻³, the rice seeds were grown from the very beginning under oxygen-free conditions. This excluded not only the possibility of functioning of mitochondria but, probably also, their normal formation. One could therefore suppose that such mitochondria as yeast promitochondria^{7,8} do not contain all the carriers of the respiratory chain and possess an enhanced resistance to anaerobiosis. High resistance of cell ultrastructure to anoxia could be, on the other hand, secured by intensive energy output through glycolysis.

With this hypothesis in mind, we were interested to find out whether the cells of coleoptiles would lack resistance to anoxia after their mitochondrial apparatus and the whole system of aerobic metabolism had already begun to function under conditions of normal oxygen supply. For this purpose rice (*Oryza sativa*) seeds were cultivated from the beginning with a normal air supply; only after 6 d of growth were the plants placed in anaerobic conditions for 24, 72 and 120 h.

After corresponding exposure to an atmosphere of nitrogen (99.997% purity) sections of the lower part of coleoptiles, of leaves and of roots (1-2 mm from the tip) were fixed by the method of Karnovsky⁹, embedded in Epon 812 and studied under a Tesla BS-513 electron microscope.

Destructive changes in mitochondria and other organelles in roots take place 1-3 d after they have been transferred to anaerobic conditions (Fig. 1a, b). This did not occur with coleoptiles. Their mitochondria and other organelles remained intact even after 120 h exposure to the nitrogen atmosphere. Some of the mitochondria remained intact, without any substantial changes in form and size, producing only more regularly ordered cristae. Other mitochondria

were transformed to large organelles with multilayer cristae. These organelles often had an irregular form (Fig. 1d). They

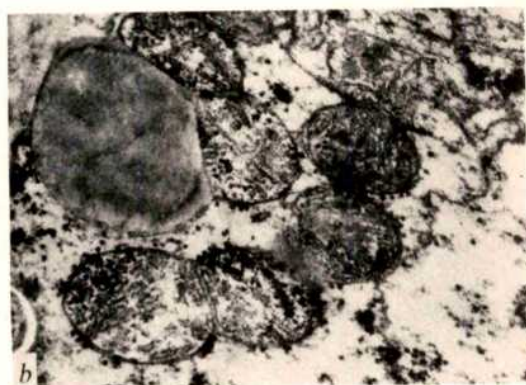
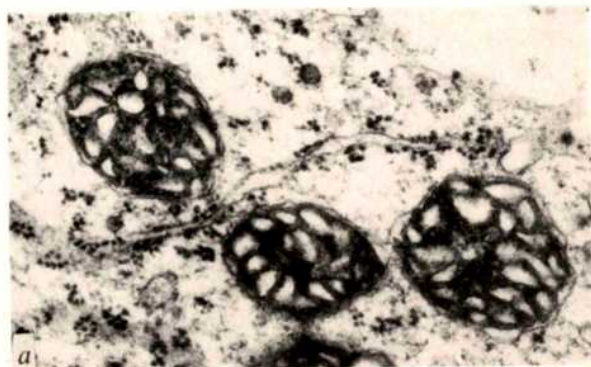


Fig. 1 Ultrastructure of mitochondria in cells of rice roots and coleoptiles in aerobic and anaerobic conditions. *a*, Root, 9 d after growth in aerobic conditions; *b*, root, 6 d of aerobic growth, then 3 d of growth in anaerobic conditions; *c*, coleoptile, 9 d of aerobic growth; *d*, coleoptile, 6 d of aerobic growth, then 5 d of growth in anaerobic conditions.

were 1.2–1.5 μm long whereas normal mitochondria are 0.5–0.6 μm long. The whole interior chamber of these mitochondria was filled with densely packed parallel cristae lying transversely to the long axis of the organelle.

Such mitochondria have not, as far as we know, been described yet in plant cells, for which layers of parallel cristae are not characteristic¹⁰. They are found in animal cells performing especially active work (cells of flight muscles in insects, myocardium of mammals)¹¹.

The appearance of these mitochondria, extremely unusual for plants, can hardly be attributed to the beginning of organelle degradation; it rather points to hypertrophy of the mitochondrial apparatus, arising in rice coleoptiles cells because of shortage in oxygen supply.

It is interesting that the mitochondria of leaves, which in contrast to coleoptiles do not start growing in the absence of O_2 revealed unexpectedly high resistance to anoxia: 3–5 d exposure of plants to nitrogen did not lead to their destruction.

High resistance of rice coleoptile cells to anaerobiosis is thus reflected not only in their ability to preserve mitochondrial apparatus when growing in strictly anaerobic conditions but also in especially active development of a number of mitochondrial cristae, possibly promoting more complete extraction of traces of oxygen from an environment which almost completely lacks it.

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Dynamics of a brachiating siamang [*Hylobates (Symphalangus) syndactylus*]

THE slow brachiation which is characteristic of the siamang [*Hylobates (Symphalangus) syndactylus*] and to a lesser extent the smaller gibbons [*Hylobates (Hylobates) spp.*] is often described as pendulum-like^{1,2}. This comparison is apt because both the pendulum and the gibbons rotate about a fixed overhead fulcrum and make use of gravitational acceleration to effect movement. Beyond these basic observations, there have been few attempts to elucidate the mechanical aspects of this unique form of locomotion^{2,3}. Analysis of 16 mm cine films of free-ranging and captive siamangs and gibbons reveals details of their locomotor behaviour that can be interpreted as precise adaptations for maximising the forward momentum gained from pendular movement.

This study is based on excerpts from several thousand feet of 16 mm film of wild siamang in Pahang, Malaysia and 100

feet of 16 mm film of captive siamang and gibbons on semi-natural islands in the Zoo Negara, Malaysia. Film was shot by the author with a Beaulieu R16B camera at speeds between 24 and 64 f.p.s.

The slow brachiation of the siamang involves a repeated series of movements. A representative cycle (Fig. 1) can be analysed in terms of two distinct phases involving the coordinated movements of trunk and legs as well as arms. The downswing phase begins as the animal releases the trailing hand. As the body swings forward and down with the legs extended, the free forelimb remains extended and sweeps through an arc behind the body. The upswing begins as the siamang flexes both legs and the free forelimb; midway through the upswing the forelimb is extended for the next grasp. During a complete swing, the trunk rotates through approximately 180° about a vertical axis.

In this form of locomotion, there are three ways in which the siamang can maximise its forward momentum: by maximising the change in kinetic energy on the downswing; by minimising the loss of kinetic energy on the upswing, and by minimising any lateral components in its momentum. Analysis of the siamang's posture in different phases of a single swing shows how the postural changes are adapted to maximise forward momentum in all three ways.

The total kinetic energy, and hence the maximum velocity acquired during the downswing, is proportional to the distance between the animal's centre of mass and the centre of rotation (in this case its hand). In a frictionless system, the increase in kinetic energy is proportional to the change in height of the centre of mass during the downswing ($\frac{1}{2}mv^2 = \frac{1}{2}lw^2 = mg\Delta h$). For a given arc (α) of

rotation, the vertical displacement of the center of mass increases with the distance between the centre of mass and the centre of rotation [$\Delta h = \Delta r(\sin \alpha)$]. The siamang increases this distance on the downswing by extending its legs and its free arm (Fig. 1).

On the upswing, the siamang minimises the loss of momentum due to gravitational deceleration by approximating its centre of mass to the centre of rotation. This is accomplished by flexion of the legs and the free arm (Fig. 1). By decreasing the moment of inertia, this redistribution of mass should increase the rotational velocity of the siamang. This increase in velocity can be seen in stop-frame analysis.

For all of the kinetic energy acquired on the downswing to be converted into forward momentum, the centre of mass should travel in the same vertical plane as the centre of rotation. The siamang is able to limit lateral motion of the centre of mass between handholds by extensive rotation at the wrist, elbow, and shoulder (Fig. 1b).

In the motion of a simple pendulum all of the kinetic energy acquired on the downswing is subsequently changed to potential energy on the upswing so the pendulum retraces its path indefinitely. In a single swing of the siamang, the kinetic energy lost on the upswing is less than that acquired on the downswing because of the input of potential energy resulting from the redistribution of mass on the upswing. By the mechanisms described above, the siamang could theoretically acquire a net momentum from each swing.

In most forms of locomotion, an animal must exert a propulsive force against the environment in order to acquire a forward momentum. Although the brachiation of the siamang as described above probably involves some energy expenditure in supporting the body, only with the rotation of the trunk about the hand is there a suggestion of any work being performed against the superstrate. In instances when siamangs do not utilise this pumping mechanism, active rotation and brachial flexion appear to become more important. Further studies involving electromyography could possibly determine the nature of the muscular activity involved.

The analysis above illustrates a way in which a slowly brachiating siamang could maximise his net forward momentum from gravitational acceleration without exerting any propulsive force against the environment. In the same way that a child on a swing can increase the kinetic energy of that system by 'pumping', the siamang is able to maximise the kinetic energy of a single cycle by appropriately timed redistribution of its mass.

This work was supported in part by grants from the Hooton Fund, William F. Milton Fund and a biological training grant from the National Science Foundation to Harvard University, and the National Science Foundation to F. A. Jenkins, jun. All work in Malaysia was done in conjunction with the Institute for Medical Research, Kuala Lumpur and the Malaysia Game Department. I thank David Chivers for introducing me to the study of Malaysian primates. I thank R. T. Bakker, D. Fisher, R. Kay, J. Peterson and especially F. A. Jenkins, jun., for suggestions, comments and general encouragement and Laszlo Meszoly who prepared the illustrations.

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a



b

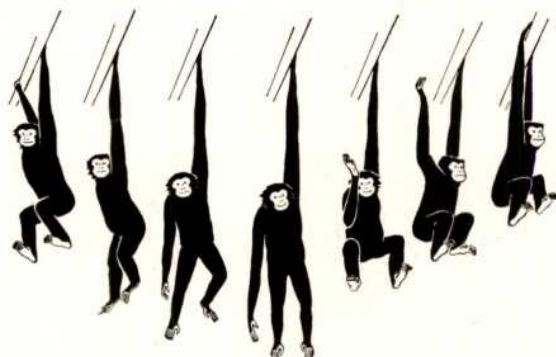


Fig. 1 a, Lateral view of a brachiating siamang, b, anterior view of a brachiating siamang. Figures rendered from tracings of 16 mm film stopped at intervals of $\frac{1}{30}$ s.

book reviews

Metamorphic rocks

Metamorphism and Metamorphic Belts. By Akiho Miyashiro. Pp. 492. (Allen and Unwin: London, September 1973.) £8.60.

THIS book is a welcome addition to geological literature; it gives a clear and positive approach towards an understanding of metamorphism, metamorphic belts and associated phenomena, and summarises much of the relevant literature.

The book is divided into three parts, of which the first presents basic concepts and methods of metamorphism and metamorphic petrology, together with a chapter where some major problems of metamorphic geology are objectively discussed. The second and third parts respectively concentrate upon two major aspects of metamorphic studies: the detailed examination of minerals and mineral assemblages, important to an understanding of petrogenesis; and the broader study of the geological relations of metamorphic terrains of various types, whence the nature and conditions of major tectonic processes and crustal evolution may be elucidated.

Part 2 summarises much descriptive literature on numerous sequences of progressive metamorphism throughout the world, and discusses their interpretation with regard to the compositional and reaction relations of minerals and mineral assemblages. Attention here is particularly focused on the abundant data available for regionally metamorphosed pelites (actually referred to as metapelites!), metabasites, and metaclastics; but there are also chapters on meta-limestones and contact metamorphism, together with a lucid account of the relations of eclogites and the eclogite facies.

In part 3, Miyashiro describes the general nature and distribution of regional metamorphic belts in North America, Europe, Japan and the Southwest Pacific. This is followed by chapters summarising the main features of metamorphic belts and examining their place in the tectonic, geochemical, and major petrological evolution of the Earth's crust. There is a particularly valuable summary and discussion of metamorphism and island arcs in the Japanese Islands. Many features are discussed in relation to the recent concepts of seafloor spreading and plate

tectonics, and these are shown to allow much interpretation of the origin of paired metamorphic belts. Much clearly remains to be learned about other metamorphic belts. Chapters on ocean-floor metamorphism and cataclastic metamorphism are included at the end of part 3; the former is a welcome addition to the usual ambit of metamorphism, but the latter is very perfunctory.

The division of the book into the three parts outlined above is a useful and logical one, but has led to some repetition and the presentation of some subjects (such as facies and facies series) in two sections. Chapter 3, in part 1, is almost a summary of the whole book. An appendix on the "History of the Study of Metamorphism", helpfully puts different approaches and viewpoints in perspective; Miyashiro does not do justice to his own major contributions.

The facies classification is used principally as a basis for the major subdivision of the P-T field of metamorphism, and Miyashiro adopts Eskola's eight facies together with the recently widely recognised zeolite and prehnite-pumpellyite facies. He laudably avoids erecting further facies which cannot be clearly and positively defined by paragenetic relations and which are unlikely to be adopted by the non-specialist—the use of petrogenetic grids is indicated for detailed facies subdivision. At the same time Miyashiro distinguishes, somewhat anomalously, some subfacies on the basis of facies series, even though paragenetic criteria may not be available. By placing the emphasis upon individual progressive grade sequences, broadly separated into three basic types but without detailed subfacies division, the approach adopted contrasts with that of the recent books by Turner (McGraw Hill, 1968) and Winkler (Springer-Verlag, 1967) where divisions into facies and/or subfacies form major aspects or objectives of presentation. This feature, together with the extensive treatment of the broader geological relations of metamorphic terrains, will probably make Miyashiro's book the most appropriate and readable one for many undergraduate teaching courses.

Miyashiro notes that he has not intended any advanced treatment of thermodynamic and structural aspects. Summaries are presented for the ther-

modynamic characteristics of metamorphic reactions, the significance of isograds, and the nature of petrogenetic grids and Schreinemaker's bundles; but they are often too condensed to greatly help those who are not already aware of their import. Little attention is given to detailed petrography, or the relations of deformation and mineral growth. Miyashiro uses the old, rather vague, geological meaning of recrystallisation, instead of the more specific metallurgical one, recently employed by many metamorphic petrologists.

This book should prove useful not only to students and research workers studying metamorphism, but also to all those interested in the evolution of the Earth's crust.

B. HARTE

Desert plants drinking

A New Ecophysiological Approach to Forest-Water Relationships in Arid Climates. By I. Gindell. Pp. 142. (Junk: The Hague, 1973.) 40 guilders.

THE title of this book constitutes a claim to originality and that claim is amply justified within its pages. Gindell has taken a refreshingly novel look at the water relations of arid land plants. He has selected each of the anatomical and physiological properties which are normally associated with xerophytism, has systematically demolished entrenched assumptions and has constructed in their place a new and very personal philosophy of the xerophyte.

Some aspects of this reconstruction are more convincing than others. For example, he shows that many xerophytes have as many stomata on their upper leaf surfaces as they do on their lower surfaces; contrary to expectation, some xerophytes open their stomata during the night; following the exhaustion of available soil water, some xerophytes maintain turgour by the absorption of mist and dew, particularly during the night. Less convincing is Gindell's suggestion that water and even ions absorbed by stomata can be transferred to the soil through the roots, thus maintaining soil moisture levels beneath vegetation. He has not dealt adequately with the alternative explanation which relates the higher soil moisture beneath vegetation to the modified microclimatic conditions in such situations. Other, broader issues, such as overall transpiration rates in

xerophytes and even the function of transpiration in general are discussed, but no firm conclusions are reached.

The book is not easy to read. The language lacks fluent style, and, worse, many interesting thoughts are buried in a plethora of rather trite information. Often it is difficult to distinguish between the ideas for which Gindel has some experimental data and those which are purely speculative. The diagrams are embarrassingly crude and misspellings and misprints common.

In general, one is left with the impression that the book was prepared and produced in a hurry, which is sad because many of the ideas which are presented in it are stimulating and iconoclastic.

PETER D. MOORE

Life in space and time

Biogeography: An Ecological and Evolutionary Approach. By C. B. Cox, I. N. Healey and P. D. Moore. Pp. viii+179. (Blackwell Scientific: Oxford, 1973.) £3.00.

THIS is a very attractive book which is well illustrated and written in a lively and engaging style, so that it is likely to appeal to students at universities for whom it seems to be intended. But as with so many new textbooks, it tends to skim the cream and omit much of the milk from which the cream rises. It is fair to ask if such books are a proper diet on which to nurture a critical appreciation of an essentially descriptive and analytical subject, for this is surely what biogeography really is. It is the branch of science concerned with the study of distribution on a geographical scale of both fossil and living organisms and the communities and ecosystems of which they form a part. It cannot be conceived as including ecology and cytogenetics without destroying the meaning of words.

The authors have too broad a definition of their subject, at least when attempting to present it so summarily. With only 179 pages at their disposal, which includes numerous maps, drawings and photographs, the treatment is inevitably often superficial, even to the point of being misleading. A model of what is really needed for universities is provided by Stanley Cain's *Foundations of Plant Geography* (Harper, New York and London, 1944), an outstanding contribution to biogeography which is surprisingly not even mentioned in the references. In fact, the chapters on life on small islands and on continental drift approach this quality and present lucidly both the essential evidence and the arguments so that they serve to emphasise the weaknesses in other chapters. For example, the account of

soils is unbalanced. What is clearly a podzol, though this is not stated, is described on page 49 and gives an impression that most soils conform to this basic structure. The process of podzolisation is described subsequently. Surprisingly in a book of this title, there is no account of the great latitudinal belts of zonal soil types and no discussion either of the relationship between these and the major ecosystems of which they are a part, or of the conceptual parallelism between zonal soils and vegetational climax. Surely if a book must be so concise, it is these fundamental relationships and ideas which should be included and illustrated with a few carefully selected and adequately presented examples. Moreover, examples must be recognisable as such and not be mistaken for generalities. In fact, this technique is used in the chapter on small islands and its value is at once apparent.

There are a few confusing statements and discussions. On page 16, *Arbutus unedo* is correctly stated to grow "wild" in western Ireland, yet on page 17 its morphology and phenology are given as evidence that it is "not native". Could the same be said of *Hedera helix*? The discussion of interaction of factors and limiting factors on page 30 suggests a fundamental misunderstanding in the authors' minds. The text is almost free of trivial errors but the caption on Fig. 17 implies a knowledge of German which is scarcely appropriate for those who aspire to be the heirs of Alexander von Humboldt.

C. D. PIGOTT

Alpha rhythm from eyes

The Origin of the Alpha Rhythm. By O. Lippold. Pp. ix+267. (Churchill Livingstone: London and Edinburgh, 1973.) £6.

THE alpha rhythm of the electroencephalogram consists of electrical oscillations at about 10 Hz which can be recorded from the scalp, especially the occipital scalp, in most people during most of the time that their eyes are in darkness. Since the work of Adrian and Matthews in 1934 it has been almost universally believed that it is generated by the brain. Dr Lippold proposes the revolutionary idea that it is generated by muscular tremor in the orbits. The evidence comes chiefly from the following three observations. (1) A tremor of the eyes can be recorded which correlates fairly well with the alpha waves. (2) If the amplitude of the corneo-retinal potential is made different in the two eyes by dark-adapting one eye, the alpha waves from the occiput become larger on the side where the corneo-retinal potential is larger. (3) If one orbit is cooled and the other warmed (thereby presumably raising the

tremor frequency on the warmed side and lowering it on the cooled side), the alpha waves become of higher frequency on the warmed than on the cooled side.

If these observations are strictly and regularly true, I do not think that Dr Lippold's conclusion can be escaped. I therefore eagerly await their independent confirmation.

The implications of the conclusion for the conduction of current within the brain are remarkable. It is somewhat surprising that electrical waves generated in the orbit can appear much larger in records from the occipital than from the frontal scalp. It is much more surprising that they can be very large in direct bipolar recordings from the occipital cortex and undetectable in direct bipolar recordings from the precentral gyrus, only a few centimetres away and slightly nearer to the orbital fissures and optic foramen. Yet this, according to Jasper, is true of alpha waves.

G. S. BRINDLEY

Through the Earth

Physics of the Earth and Planets. By A. H. Cook. Pp. x + 316. (Macmillan: London and Basingstoke, September 1973.) £8.50.

THE author states that this book was written for second and third year undergraduate geophysics students and as background reading for graduate students. He defends the bias given to such topics as gravity, seismicity and certain dynamical behaviour of the planets on the grounds that present day students know more modern than classical physics. But the bias is rather narrower than this and the author seems to be only really sympathetic to those parts of earth science that make great play with displays of significant figures. How else is one to interpret the sentence (page 33) "The shape of the solid surface of the Earth is very irregular and of no general scientific interest"? Although there are chapters devoted to magnetism and crustal movement and some extremely sketchy descriptions of apparatus, my overall impression is of a book that could serve as an introduction to Sir Harold Jeffreys's *The Earth*.

There is a good introduction but later, in perhaps more important matters of detail than the consistent use of SI units, the standard of presentation becomes less meticulous than Sir Harold's. For example Laplace's equation is said to be "nothing more than a statement of the inverse square law" on page 27, whereas on page 28 that Laplacian fields are said to vary as r^{-1} . There follows a repetitious discussion of satellite motion and a treatment of geoidal shape, complicated

enough at the best of times, that is marred by sign errors. There are other indications of hasty preparation and inadequate proof reading. Why give a glossary of symbols when additional definitions (for example of m) are introduced into the text? Why give two diagrams of travel-time curves (pages 75 and 78) when one suffices and is there any need, even in a student text, to give diagrams of upper mantle velocity distributions (page 84) that conceal quite so many complexities of the observed situation?

There follow chapters dealing with oscillations and vibrations, the internal structure of the Earth (which ends with an entirely theoretical discussion of creep and the Q of materials), the radioactivity and ages of rocks and the temperature within the Earth. The latter chapter follows entirely conventional lines, based on thermal conduction theory although we are warned (page 171) that such calculations only apply to those parts of the Earth that are "rigid". Having been told in a previous chapter about the creep of rocks and that most of the mantle is at temperatures at which creep is "almost certainly important" (page 136), the student will wonder about the consistency of pursuing this particular line of enquiry. A quite erroneous description is given of phonon thermal conductivity (page 174), valence bands are described as localised electron states (page 175) and exciton energy transport is said to dominate other modes of the heat conduction process (page 176). A completely garbled account is given of the fundamentals of thermal convection theory, and the matter is not clarified by a quite incomprehensible fig. 7.11. I was very irritated by the implication that all is still confusion and ignorance in such an important problem of planetary physics, where definite progress has been made in the past ten years.

The remaining chapters deal with the permanent magnetisation of rocks, the geomagnetic field, movements of the Earth's crust and "The Earth among the Planets", the latter being largely concerned with the constraints placed on the density distributions in planets by classical astronomical observations and theoretical equations of state. Little reference is made to the new insights that have sprung from the space programme.

It is perhaps no criticism of a book intended for students that there are so few actual references to the work of living earth scientists, although a few names are dropped from time to time. Perusing the biographical notes at the end of the book, a student could be forgiven for believing that planetary science was the exclusive preserve of titled British scientists and finished with

the death of that well known geophysicist, Lord Rutherford. He will however find the questions at the end of most chapters a challenge to his understanding—even if they are a little contrived.

Since the publishers have performed their side of the production so satisfactorily, it is a pity that so much is unsatisfactory in its contents. I fully appreciate how difficult it is to be the expert in all branches of planetary science that such a title demands, but a little more care in preparation could have made this a much better book.

D. C. TOZER

Too brief a tale

A Revolution in the Earth Sciences: From Continental Drift to Plate Tectonics. By A. Hallam. Pp. vii+127. (Clarendon: Oxford; Oxford University: London, April 1973.) £4 boards; £1.75 paper.

THIS is a narrative historical account of the concept of continental drift. It is written in much too short a space to tell a story so deeply enmeshed in personality and full of drama. Dr Hallam deliberately aims to write "quite sparingly" and his book thereby takes on an encyclopaedic character. All the principal papers are described, though often in no more than abstract form, but the background is largely missing. He tells what was written, but not what was thought. Nor does he explain the extent of knowledge and ignorance at crucial times in the narrative.

Dr Hallam has painted a stark and static picture of the players. He has barely painted the scenery at all.

DAVID DAVIES

Substitutions

Aliphatic Nucleophilic Substitution. By S. R. Hartshorn. Pp. viii+172. (Cambridge University: London, November 1973.) £3.80; \$12.50.

THIS book is a useful member of the series of chemistry texts published by the Cambridge University Press and is certainly worth a place in any college or university library.

The author has given a clear account of nucleophilic substitution; the various methods of determining the type of reaction are discussed in detail and the problem of the "borderline" cases emphasised, an important feature since many undergraduates fail to appreciate this problem and believe that all substitution reactions fall into clear-cut categories. I was very pleased to see a section on the catalysed reactions, as the effects of silver and mercury salts on these reactions are often ignored.

In other places the treatment seems a little thin: for instance, eight pages of the book are devoted to secondary deuterium isotope effects and yet ambient nucleophiles only merit half a page and Kornblum's name does not appear in the list of references. Some space should have been used to discuss the important synthetically useful reactions and their limitations: dozens of graduates go out into the world each year believing that *t*-butyl bromide plus sodium malonate ester constitutes a useful synthetic reaction. In the index there is no reference to diethyl malonate or β -keto esters and the list of solvents does not include dimethyl sulphoxide.

A. S. BAILEY

Techniques for structure

Co-ordination Chemistry: Experimental Methods. By K. Burger. English translation edited by Ian T. Millar and D. W. Allen. Pp. 372. (Butterworth: London, 1973.) £10.

TITLES must be brief, so may be incomplete. This book describes experimental methods for providing structural information in coordination chemistry and does not cover measurements of equilibrium constants or the kinetics of complex formation. In a masterly introduction the author sets the perspective and in his final, the eleventh chapter, he shows with examples from glyoxime complexes how the various methods can be combined.

The intervening chapters each deal with specific techniques giving the principles, an outline of the methods and the type of information obtainable. The balance has been influenced partly by the length of time for which the technique has been available; Mössbauer spectroscopy has advanced so much since the Hungarian edition was published in 1967 that the chapter on it was rewritten and is now as long as that on IR and Raman spectroscopy despite their more general applicability. Post 1967 references are rare in the rest of the book, and the new technique, electron spectroscopy for chemical analysis (ESCA), provides most of these.

There is a particularly clear explanation of shielding effects in the chapter on nuclear magnetic resonance (by L. Brajer). In the first three sections on X-ray structure analysis (by Korecz) there is confusion between atoms and lattice points while the illustration of an electron density map, also on the dust jacket, is not of a coordination compound; Burger himself writes well on the results. An interesting account of thermal analysis (by G. Liptay) shows that this must be combined with other methods if structural information

is sought. The use of optical rotation and circular dichroism in stereochemistry are well described and the account of magnetic methods presents spin-orbit coupling with unusual clarity.

The book succeeds in its aim, to enable someone starting research to choose an appropriate technique for the problem. It is also readable and sufficiently clear to help someone struggling with obscurities in a more specialised text. The references and examples cover a wider range than the monolingual scientist usually meets, as shown by the author index which, with a subject index, is another good feature. The standard of printing and illustration is good; unfortunately few research students will be able to afford their own copies.

MARY R. TRUTER

Neurones connecting

Developmental Neurobiology of Arthropods. Edited by D. Young. Pp. vii+268. (Cambridge University: London, December 1973.) £5.60.

THE editor of these nine experimental and review contributions has wisely declined to erect principles or to look too hard for coherence within the phenomena covered by the volume. Such treatment might have exerted an intellectual 'closure' effect on a field involving many young as well as more experienced workers. Instead, the impression is preserved that contributors are usually defining questions which may in future be posed, using their experimental systems. The book is thus good reading for intending researchers and teachers, and not recommended for those in search of potted literature from which to bolster grand theory.

Readers with developmental interests, and vertebrate research experience, spend much of their time fascinated by features distinctive to arthropod as opposed to vertebrate systems, particularly the relatively small number of neurones, and the high proportion of these which seem to be uniquely specified and 'homologous' anatomically as between individuals. But they are continually reminded that the anatomical picture of a lower order of determinacy within vertebrate nervous systems does not itself rule out a comparable degree of cell specificity at some functional level. Thus arthropods may lend themselves well to developmental analysis while contributing greatly in future to knowledge of general principles underlying neural organisation, where such exist. Both aspects are stressed at points in the book. The concluding review by Horridge contains discussion of uniqueness among neurones, and the editor in his own contribution makes some very thought-provoking remarks (page 186)

on the homology concept as applied to cells within successive segmental ganglia.

Levi-Montalcini and her associates provide a rich source of technical information for initiates into insect nervous system tissue culture, together with evidence for specificity capacities of growing neurites that has so far emerged from such work.

Bate and Lawrence review the theory, concerning spatial organisation in insect development generally, with which the latter is associated, and then suggest its relevance to such organisation as studied in the eye-brain connections of vertebrates. They then show how a particular piece of specificity in sensory behaviour, emerging in an insect pupal stage, is explicable if different receptor cell-bodies form central connections according to their position in a quantitative antero-posterior gradient of positional information that controls epidermal pattern formation in general. In such a cross-disciplinary publication as this they might have discussed alternative theories available for positional information control, but the important point is made that if position within a cell array is signalled quantitatively only, then competitive mechanisms are strongly implicated in normal erection of neuronal projections. Edwards and Palka in fact offer evidence of synaptic competition during development, in their chapter on sensory regeneration.

The insect eye—optic lobe system, described by Meinertzhagen, deserves detailed acquaintanceship as perhaps the largest array of neurones known in which many are completely specified as to anatomical class and connectivity. Although making demanding reading, this chapter is perhaps the most exciting for the relative newcomer to arthropod neurobiology.

Pipa and Bentley each provide surveys of post-embryonic development, from functional and anatomical viewpoints, and Hoy introduces the rather outlandish properties of regeneration and survival in certain crustacean axons.

JONATHAN COOKE

Associates of viruses

Viruses and Invertebrates. Edited by A. J. Gibbs. Pp. xvi+673. (Frontiers of Zoology vol. 31.) (North-Holland: Amsterdam and London; American Elsevier: New York: 1973). Dfl 150; \$57.70.

THE editor of this book believes that it is vital to develop a unified approach to virology, to counteract a bigoted adherence to an order of events based on the taxonomic position of the host. This philosophy deserves active support. The topic of 'viruses associated with invertebrates' was a particularly good choice as it allows attention to be focussed on

both animals and plants. The enormous scope of the subject is however, demonstrated by the fact that the editor needed the assistance of thirty-three authors who contributed thirty-one chapters. It is understandable that, with so many collaborators, not only will variable standards of treatment and presentation arise but that dangers of superficiality and irrelevance may occur. But with one or two exceptions, Dr Gibbs has kept his troops under disciplined command and produced a valuable and stimulating book.

The names of the various sections owe more to the stage and art than to science and yet they stimulate interest in the contrasts and direction of thought contained within and between the various chapters. For example, to continue with the artistic analogy, "Tryptych" leaves an impression of a miniature dominated by two massive canvasses. The quality of the paper is poor: it is so thin that photographs on the reverse side of a page become obtrusive. Also, a general index of only two pages is derisory and one has to rely on the brief contents list of each chapter. The price of the book is high and it will probably only find its way into libraries, but it is worth borrowing.

T. W. TINSLEY

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Is space time?

Space and Time in the Microworld. By D. I. Blokhintsev. Pp. xiv+330. (Reidel: Dordrecht and Boston, Mass., 1973.) Dfl. 110; \$39.50.

BOTH relativity and quantum theory profoundly alter Newtonian concepts of space and time. Each theory introduces changes that are qualitatively different and their compatibility has been the topic of many debates. In special relativity, the individual process is still analysed in terms of point particles moving along trajectories in a manifold, although the interactions are always propagated with a bounded finite velocity. Quantum theory, on the other hand, only gives meaning to the individual process in terms of the ensemble and, furthermore, there seems to be an essential connectedness between interacting systems that are eventually spacially separated, giving rise to a correlation that seems to be non-local.

In the non-relativistic domain we know how to accommodate the quantum theory; the classical analogue of the phenomena can be successfully translated into the quantum algorithm, but once we move into the high energy region, the situation becomes somewhat confused. Does the essential connectedness of quantum theory imply that space-time descriptions are futile and that Lorentz invariance should be introduced through energy-momentum considerations alone or should we ask questions about possible modifications to space-time geometry? Indeed, the aim of Professor Blokhintsev's book is to present a critical analysis of this type of question.

He begins by developing the mathematical description starting from the Minkowski formalism of space-time, passing briefly through field theory, finally arriving at the S-matrix formalism which is to form the basis for the rest of the investigation. In fact, he goes as far as to claim that the S-matrix will be retained in all future theories, although the methods of constructing the matrix elements may undergo radical changes.

His main thesis then centres around the question of causality. In particular, he considers the consequences of replacing microscopic causality by a less restrictive form of macroscopic causality. This allows some form of acausality, or non-locality, in small space-time regions, while retaining causality in the large. The S-matrices constructed using either principle of causality differ only at energies that are much greater than the reciprocal of a fundamental length. Experiments show this to be less than 10^{-14} cm and the author speculates that it could be 10^{-16} cm, the characteristic

weak-interaction length. But the arguments are slender and it could equally well be at 10^{-33} cm, the gravitational length, in which case further investigation might be discouraged. It has, however, often been suggested that the infinities in both classical and quantum field theories could be removed by changing the geometry in the small and the advantage of the principle of macroscopic causality is that it allows an exploration of various possibilities without leading to large-scale acausality. The final chapters are concerned with this question and discuss, in particular, the effects of discrete space-times, stochastic space-times and fluctuating geometries.

These final chapters left me feeling a little disappointed that more space had not been given to the question of generalised geometries. I find this a very fascinating field in which many questions are still unanswered. Indeed there is quite an extensive literature on the subject some of which is quite closely related to the topics discussed in the book but unfortunately no reference is made to this work. Apart from this small reservation, I find the book very clearly written, and even the liberal use of mathematics has not been allowed to obscure the essential questions.

B. J. HILEY

All sorts of skin

The Integument. A Textbook of Skin Biology. By R. I. C. Spearman. Pp. vi+208. (Biological Structure and Function, vol. 3.) (Cambridge University: London, June 1973.) £4.20; \$12.95.

BECAUSE of the explosive developments taking place in science in general and biology in particular and because of the trend toward more and more specialisation, books which give a broad, comprehensive view of the subjects included under these headings have become a rarity. Thus, I began to read this book with some anticipation which was further whetted by the author's commitment to provide a bird's-eye view of the whole subject of skin biology. As the author states in his preface, the comparative approach to the study of the outer covering of both invertebrates and vertebrates has not been attempted before; hence any book purporting to do so should be expected to fill a long-felt need. Unfortunately, *The Integument* fails to live up to these expectations and does little more than catalogue the various aspects of skin biology under various headings.

The book is divided into two parts, the first of which treats the comparative morphology of the integument of both invertebrates and vertebrates. Here

Spearman deals with the epidermal, dermal and pigmentary aspects of the integument of various phyla of invertebrates and of five classes of vertebrates (Pisces, Amphibia, Reptilia, Aves and Mammalia). This section is nicely illustrated with numerous line drawings which should help the beginner to understand the structural details of many of the skin appendages.

In the second part, the book deals with the comparative functions of skin, such as thermal regulation, chemical and neural control mechanisms, comparative synthetic processes, transport through the skin, immunity and development. The titles are provocative, but the cursory treatment leaves much to be desired. For example, in the chapter on the comparative synthetic process, only ten pages are devoted to such varied topics as genetic code, protein syntheses, elastin, resilin, collagen, and a host of others.

Brevity may be the soul of wit, but since wit is hardly the reason for undertaking a study such as this, the kind of one-sentence treatment accorded to important topics by Spearman while serving to whet the appetite does little to allay the hunger for substantial data.

PAUL PARAKKAL

Kidney disease

Renal Histopathology: A Light Microscopy Study of Renal Disease. By Robert Meadows. Pp. xii+363. (Oxford University: London and New York, December 1973.) £14.

WITHIN the limits of the title Dr Meadows has written an interesting and very well illustrated book. It will be useful to all pathologists who examine renal biopsies and to the many physicians who seek to understand the structural changes in their patients. While agreeing with the author that most diagnoses can be achieved with the light microscope, fortified by knowledge obtained by others with ultrastructural and immunofluorescent methods I feel it is a pity that there is not more reference to these in the text and some illustrations of the immunofluorescent results which after all are obtained with the light microscope.

The consideration of glomerular changes and glomerulonephritis is comprehensive and clear although the separation of the two leads to a certain amount of recapitulation. The section on the disappearance of glomeruli from the kidney is welcome in that it considers a difficult point which most authors skate over. Dr Meadows is not alone in attributing the clear description of what he calls the ischaemic obsolescent glomerulus to McManus (1950); it was in fact recognised by Russell (1929) by the

less cumbersome term of ischaemic atrophy. She used the term toxic atrophy for a glomerular lesion in chronic glomerulonephritis which is redescribed in more detail by Dr Meadows who puts forward the thesis that some cases of chronic glomerulonephritis develop as a progressive form of focal disease. In her Medical Research Council Special Report (No. 142), Russell (1929) had an interesting name for this concept—nephritis repens—which has never caught on. Perhaps these reports repose in dusty basements or galleries of libraries in Australia just as they do in this country.

The chapters on tubular disease are not quite as good as those on glomerular because of a lack of adequate integration with experimental work. In understanding the changes in biopsy and necrosis material from acute tubular necrosis, for example, it is essential to appreciate the appearances of regenerating tubular epithelium at various stages—the details of which are most easily seen in experimental mercury poisoning. The only consideration of such regeneration is confined to ischaemic tubular necrosis and the sequence is not brought out. The part which obstruction may play in the pathogenesis of tubular damage is not discussed; in this respect it is unfortunate that the paper of Bywaters (1946) in which he brought out the resemblance between changes in acute hydronephrosis and the crush syndrome has never received proper recognition.

J. F. SMITH

Pictures of viruses

Ultrastructure of Animal Viruses and Bacteriophages. An Atlas. Edited by A. J. Dalton and F. Hagenau. (Ultrastructure in Biological Systems vol. 5.) Pp. xii+413. (Academic (Harcourt Brace Jovanovich): New York and London, September 1973.) \$37.50.

THIS book contains a splendid collection of electron micrographs illustrating the structure and development of 65 animal viruses and bacteriophages. Though the text is variable from chapter to chapter it is mostly concisely informative. The index is very comprehensive but it did not include the Sendai virus, which is now much used in cell fusion studies.

The chapters are divided according to the virus groupings laid down by the International Committee on Nomenclature of Viruses (ICNV). Each begins with a concise account of the structure of the virion and of its intracellular replication. As might be expected the text is not a comprehensive treatise on each group of viruses nor is each group uniformly treated. For instance R. W. Horne and B. Roizman each devote nine pages to their respective groups the adenoviruses and the herpesviruses,

whereas N. Higashi and A. J. Dalton fill only one page each about the togaviruses and the arenaviruses. Nevertheless all the chapters have useful lists of references.

Several authors have been particularly helpful by giving clear accounts of more obscure aspects of their chosen group. A. J. Dalton and F. Hagenau define the A, B and C particles found in neoplastic tissues and G. C. Goodman gives a detailed account of the cytopathic events in cells infected with picornavirus in the captions to the illustrations. J. L. Melnick gives an account of the efforts of the ICNV to name and classify the viruses.

It is a pity that a book which treats the animal viruses so fully should expend two chapters on bacteriophage but exclude the viruses of plants and invertebrates. But the book completely achieves its editors' aims, provides a valuable work of reference for the animal viruses and gives a concise view of the bacteriophages.

D. KAY

Cosmic rays reach earth

Cosmic Rays at Ground Level. Edited by A. W. Wolfendale. Pp. vii+232. (Institute of Physics: London and Bristol, 1973.) £12.00.

As a tribute to Professor G. D. Rochester on the occasion of his retirement, nine authors associated with the cosmic ray group at the University of Durham have chosen to write ten short reviews, in which they have summarised the present situation in experimental work on the fluxes of the various components of the secondary cosmic radiation detected at "ground level", in chapters on energetic muons (M. G. Thompson), protons and pions (Brooke), neutrons (Ashton), μ -neutrinos (Osborne), e -neutrinos (E. C. M. Young), extensive air showers below 10^{17} eV (Wdowczyk) and above 10^{17} eV (Turver), and on searches for quarks and other hypothetical particles (Ashton). The work is not about the primary cosmic radiation, although for completeness a preliminary chapter (Wolfendale) sketches in some of its aspects. There is added a comprehensive account of the technique of observing trajectories of fast charged particles by neon flash tubes (Breare), a method used by most of the authors.

In several chapters the aim is principally to collect data on the particle fluxes near sea level, and the "ground" in the terms of reference strictly excludes mention of experiments at mountain altitudes: hence a theoretical framework for interpreting the observations is not the aspect of interest. In the circumstances it is natural that the authors, apart from collecting published data, present progress reports which tend to lean heavily

for illustration on the experiments in which they and their colleagues took part, in the research groups founded by Rochester and Wolfendale; although extensive references are given to other experiments.

In the most substantial review, on observations of momentum spectra, angular distributions and charge ratios of energetic muons, readers with a peripheral interest in the subject may be surprised by the extent to which for many years results have depended on normalisation to an old experiment by Greisen: only recently has the normalisation been revised (by Allkofer and others), and it is useful to have this documented. (It is a pity though that the "ground level" rule bars any reference to the work of the Utah group.) Although some problems currently raised by the interpretation of the charge ratio are briefly introduced, the significance of the vertical momentum spectrum is not discussed. The reviewers of hadrons have very little to work with at energies above a few GeV, mountain experiments having been more numerous, and the distinction between pions and protons (at sea level) evidently remains a problem. Here, though, a discussion of the method of deducing the energies of hadrons in the TeV range from the cascades they produce would have been welcome, as different experiments are almost certainly not using the same scale. (In the short chapter on neutrons, the method used to measure their spectrum is not mentioned at all.) And for all very high energy spectra, one would have liked to know how much accompanying particles might affect the measurements.

To observe neutrinos one has to go to deep mines, but with the excuse that the fluxes there and at sea level must be the same, Osborne gives a clear account of the three main experiments on μ -neutrinos, and their significance. Young and Ashton have no positive observations to report on their exotic particles, but they indicate what intensities might reasonably be sought. In the articles on extensive air showers the emphasis shifts to illustrating progress being made by the authors and their colleagues in constructing models of shower development, which is a necessary preliminary to firm deductions about the energies and nature of the primary particles; although Turver also presents recent estimates of the energy spectrum, and isotropy, and recent work on the distribution of several particle components of air showers, largely from the Haverah Park experiments.

The fluxes of electrons, gamma rays and slow neutrons are not reviewed. It is a pity that the price is so high for a welcome but relatively short book.

A. M. HILLAS

matters arising

T-mycoplasmas and infertility

SIR,—IN the first report about the association of T-mycoplasmas with human infertility the authors considered that the high incidence of isolation (89%) from the cervix of infertile women compared with the low incidence of isolation (23%) from pregnant women suggested that the T-mycoplasmas were playing some rôle in the infertility. This was based on the isolation discrepancy and was principally a result of the low incidence in pregnant women. Others have, however, been more successful in isolating T-mycoplasmas from pregnant women. Mårdh and Weström² isolated T-mycoplasmas more frequently from pregnant (68%), than from non-pregnant women (46%), and thought that the former rate might have been due to changes in the genital tract at pregnancy conducive to the growth of T-mycoplasmas. Gnarpe and Friberg tried to substantiate the possible association of T-mycoplasmas with infertility by treating infertile couples with tetracycline and 29% of the women they treated subsequently became pregnant. In the absence of untreated controls, however, this finding is meaningless. Mr G. T. Smedley and I, in an unpublished study, found that of 32 infertile women, 22 (69%) carried T-mycoplasmas. Within a year, nine of these women became pregnant without treatment and 5 (56%) of them carried T-mycoplasmas. Gnarpe and Friberg's second report³ concerns the attachment of T-mycoplasmas to human spermatozoa. I find it difficult to regard the 'blobs' on the spermatozoa that they saw by electron microscopy as evidence for attached T-mycoplasmas, as these structures could also be *Mycoplasma hominis*, chlamydiae or some other agent. I do agree, however, that adherence of human T-mycoplasmas occurs in the same way as those of bovine origin adhere to bovine spermatozoa⁴. It is interesting that practically all bovine semen samples that we examined contained T-mycoplasmas, yet they all had a high fertilising capacity⁵. It seems likely that abnormal appearance, low motility and viability of spermatozoa in man are not due to the presence of T-mycoplasmas in semen, because the organisms are isolated from such samples no more frequently than from samples which contain apparently normal spermatozoa. Spermatozoa of low fertilising potential might also be

adversely affected by mycoplasmas present in the vagina and cervix. This comes back to the original point about the incidence of T-mycoplasma isolation from these sites. Whether the incidence of isolation is greater in infertile women is a moot point, but it would also be interesting to know whether they harbour more organisms in the genital tract than do fertile women. I feel that the evidence put forward to incriminate T-mycoplasmas as a cause of infertility is far from convincing and that they are being placed on a pedestal that they do not, so far, deserve.

Yours faithfully,
D. TAYLOR-ROBINSON

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Harrow, Middlesex

¹ Gnarpe, H., and Friberg, J., *Nature*, **242**, 120 (1973).

² *Acta Path. Microbiol. Scand.*, **78**, 367 (1970).

³ Gnarpe, H., and Friberg, J., *Nature*, **245**, 97 (1973).

⁴ Taylor-Robinson, D., and Manchec, R. J., *Mycoplasma Diseases of Man*, Veb Gustav Fischer Verlag, Jena, p. 113 (1969).

⁵ Taylor-Robinson, D., Thomas, M., and Dawson, P. L. *J. Med. Microbiol.*, **2**, 527 (1969).

DRS GNARPE AND FRIBERG REPLY: we have read with interest the above letter and although Dr Taylor-Robinson's comments are valuable we have by no means expressed the opinion that T-mycoplasmas might be one of the major causes of infertility.

There are, however, a few points we wish to make clear. In our first investigation¹ we found a high incidence of T-mycoplasmas in infertile men and women compared with a lower incidence in men and women with proven fertility. All infertile patients had an unexplained infertility of 15 yr or more. None of the spermatozoa specimens investigated had abnormal appearance under light microscopy, low motility or reduced viability. We still consider that the incidence of pregnancy after tetracycline treatment is higher than can be expected to appear by chance among patients with this long-standing infertility².

We agree with Dr Taylor-Robinson that the 'blobs' on human spermatozoa discovered in scanning electron microscopy might be other agents such as *M. hominis* or Chlamydiae. In all specimens where cultures were made of

thoroughly washed spermatozoa³, however, we found T-mycoplasma colonies and not other agents growing. Whether Chlamydiae were present or not was, of course, impossible to establish with the technique used. We also found that some T-mycoplasma isolates produced substances cross-reacting with neuraminidase antibodies and suggested that these substances might render conception more difficult³.

These findings make it possible that some T-mycoplasmas might be of importance for some cases of unexplained infertility.

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¹ Gnarpe, H., and Friberg, J., *Nature*, **242**, 120 (1973).

² Friberg, J., and Gnarpe, H., *Am. J. Obstet. Gynec.*, **116**, 23 (1973).

³ Gnarpe, H., and Friberg, J., *Nature*, **245**, 97 (1973).

African swells, magmatism and plate tectonics

SIR,—Some of the 'flaws'¹ in my lateral compression model² seem to be matters of faith, for example, that the model is "at odds with modern global tectonics", whilst many (including the "petrological observations") are arguable matters of interpretation. One "flaw", apparently supported by data, concerns the faster rate of uplift of post-glacial regions compared with the doming of East Africa. It is argued that since there is no evidence of deep partial melting in the post-glacial regions, the probability of such melting by warping of the African craton is insignificant. But the two mechanisms are quite different. The post-glacial regions are rising in response to a uniform isostatic pressure at the base of the plate. Below the post-glacial uplift there is no localised decompression, as might be the case below an 'upwarp'. As was pointed out in 1964 (ref. 2), in the case of the African swells "lighter material would have to be added at depth to compensate each succeeding uplift". So far as I am aware, this deduction has not been challenged, nor an alternative offered.

In any case, since 1964 my original hypothesis² has been considerably modified and refined³, and Gill's critique is

therefore something of an anachronism. In particular, the interested reader will find³ the evidence and arguments against the idea (said by Gill to be "widely regarded") that the "topography of Africa is . . . the product of heat flow anomalies in the upper mantle". An alternative is proposed which is more in keeping with the facts.

Yours faithfully,
D. K. BAILEY

Department of Geology,
University of Reading

- ¹ Gill, R. C. O., *Nature*, **247**, 25 (1974).
² Bailey, D. K., *J. geophys. Res.*, **69**, 1102 (1964).
³ Bailey, D. K., *J. Earth Sci.*, **8**, 225 (1972).

Announcements

Appointments

The Council of the University of Manchester has appointed **E. R. Trueman** to be Beyer Professor of Zoology. **D. M. Guthrie** has been appointed to be a Professor of Zoology in succession to Professor Trueman.

The British Library Board has appointed **D. Richnell** as Director General of The British Library, Reference Division.

J. Latham has been appointed to a Chair in Physics and **D. S. L. Cardwell** has been appointed to a Chair in the History of Science and Technology at the University of Manchester Institute of Science and Technology.

Awards

The **Edinburgh Geological Society** has awarded the **Clough Medal** to **Dr Dorothy H. Rayner**, for her contributions to geology.

The **Zoological Society of London** has awarded the **Scientific Medal** to **Dr J. N. Brady** of the Imperial College of Science and Technology, London, and **Professor H. C. Macgregor**, of the University of Leicester; the **Stamford Raffles Award** to **Mr G. H. Locket**; and the **Thomas Henry Huxley Award** to **Dr Patricia Farquharson** of Westfield College, London.

The **Biochemical Society** has awarded the **Colworth Medal** for 1973 to **J. C. Metcalfe** of the Department of Pharmacology, Cambridge; the **CIBA Medal and Prize** to **P. D. Mitchell** of the Glynn Research Laboratories, Bodmin; and the **Keilin Memorial Medal** to **E. C. Slater** of the BCP Jansen Institute, University of Amsterdam.

International Meetings

March 28, Solids Handling and Metering associated with an NPK Prilling Plant (J. C. Pinder, The Fertiliser Society, Alembic House, 93 Albert Embankment, London SE1 7TU)

March 31–April 12, Cours de formation sur la recherche anticancéreuse (Dr J. F. Delafresnaye, Union Internationale Contre le Cancer, Rue du Conseil-Général 3,1205 Geneva, Switzerland)

April 1–2, Gene Isolation and HnRNA (Dr J. P. Goddard, Organising Secretary, Nucleotide Group Meeting, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ)

April 1–2, Synchrotron Radiation and its applications to the Analysis of Problems in Scientific Investigation (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX)

April 1–3, The Design of Scientific and Technical and Scientific Exhibitions (The Centre for Extension Studies (Z), University of Technology, Loughborough, Leicester LE11 3TU)

April 1–3, Electrets and High Field Polarization (Dr C. W. Smith, Meetings Secretary, Dielectrics Discussion Group, (Dielectrics Society), University of Salford, Department of Electrical Engineering, Salford M5 4WT, UK)

April 1–4, 5th International Geochemical Exploration Symposium (John Barakso, c/o Mineral Environment Laboratories Ltd., 705 West 15th Street, North Vancouver, British Columbia, Canada)

April 1–5, Conference on Animal Feeds of Tropical and Sub-Tropical Origin (Public Relations C, Tropical Products Institute, 56–62, Gray's Inn Road, London WC1X 8LU)

April 1–5, Basic Electron Microscopy Course for Biologists and Materials Scientists (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA)

April 1–5, Radio: Roads, Tunnels and Mines (Institut National des Industries Extractives, rue du Chera, B-4000, Liege, Belgium)

April 1–5, Post-Graduate School on Pharmacokinetics (Mr R. E. Marshall, School Secretary, Department of Pharmaceutical Sciences, The Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London WC1A 2NN)

April 1–5, International Symposium on Advances in Polymer Friction and Wear (Dr Lieng-Huang Lee, Symposium Chairman, Wilson Center of Technology, Xerox Corporation, Webster, New York 14580)

April 1–5, Annual Chemical Congress of the Chemical Society and the Royal Institute of Chemistry (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK)

April 1–6, Symposium on Afar (Ethiopia) and Related Rift-Structures (Professor A. Pilger, Geologisches Institut der Technischen Universität Clausthal, 3392 Clausthal-Zellerfeld, West Germany)

April 2–3, Sira Conference on Quality Control (Mrs Ruth Keiller, Sira Institute Ltd., South Hill, Chislehurst, Kent BR7 5EH)

April 2–4, 2nd International Symposium on Jet Cutting Technology (The Organising Secretary, 2nd ISJCT, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK)

April 2–4, The Mechanical Properties of Materials at High Rates of Strain (Dr J. Harding, Department of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PJ) (Changed from 19–21 March)

April 2–4, Multi-Phase Flow Systems (Department NTS, The Institution of Chemical Engineers, 16, Belgrave Square, London SW1X 8PT, UK)

April 3–4, Colloquium on Molluscan Phylogeny (Dr N. J. Morris and Dr J. D. Taylor, British Museum (Natural History) Cromwell Road, London SW7 5BD)

April 3–4, Metal Semiconductor Contacts 9B. (L. H. Wilson, The Plessey Co Ltd., Allen Clark Research Centre, Caswell, Towcester, Northamptonshire)

April 4–5, 547th Meeting of the Biochemical Society (Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP)

April 4–6, 6th Conference of the Institute of Information Scientists (The Conference Secretary, Mrs S. E. Stevenson, Solartron Electronic Group Ltd., Victoria Road, Farnborough, Hampshire)

April 5, Spring Meeting of the Clinical Genetics Society (Dr Derek A. Smith, Genetics Department, University of Birmingham, P.O. Box 360, Birmingham 15)

April 5–9, Franco-British Centenary Conference (The Meetings Officer, Institute of Physics, 47, Belgrave Square, London SW1X 8QX, UK)

April 8–10, National Engineering Laboratory and Society for Underwater Technology International Symposium on Exploitation of Vibration (Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 0QU)

Science and public pleasure

IN a recent interview on BBC television, Dr J. Bronowski was talking about job satisfaction. "Scientists and prostitutes", he said, "have one thing in common—they both get paid for doing something they enjoy." Many would question the enjoyment that Bronowski attributes to prostitutes but surely scientists enjoy themselves.

We looked, a few months ago, at the fun side of enjoyment—the scientific jokes of various guises—and this need not detain us here. What we now consider is the more lasting aspect of enjoyment; job satisfaction, but more than that. Some jobs offer satisfaction to the doers but hardly to others, certainly not to the general public. One does not, for instance, expect a stockbroker, however personally satisfied by his job, to look for any sort of public acclaim. At the other end of the spectrum are those who offer some sort of pleasure to the general public, not necessarily to their own satisfaction (for example, prostitutes, troubled poets and so on). Where do scientists, and in particular research scientists, fit into this scheme? There is clearly not a preordained slot in a satisfaction diagram for scientists but the question is valid, partly because recruitment into science depends substantially on how much practitioners are perceived to enjoy themselves and partly because the question of a more public delight in one's work is something about which scientists are ambiguous.

There are, doubtless, many surveys carried out among scientists of how happy they are with their job. Such surveys are of little use if they comprise no more than a question, 'Are you happy in your work?' Few will admit to being unhappy; not only is it an admission in itself but it also reveals the vulnerability of the highly trained person who has few options to move other than within his specialisation. The question 'do you give other people pleasure through your work?' is a more difficult and telling question. It is also one which will be dismissed by many as irrelevant. 'Science is not show business.' But it is, and there is nothing disreputable about show business, which comprises music as well as music hall, theatre as well as circus.

Many myths about scientists have been exploded in the past ten years and among these has been that the scientist has some sort of detachment both with regard to his personality and to his way of doing things. *The Double Helix* must surely have been the most eloquent source of this demythologising. And yet the emotions which Watson so vividly and honestly portrays are not confined to those who make the greatest of discoveries; in a sense they are even stronger among those whose names have not become household words because the final accolade is still to come (or never will). It is inconceivable that a vast majority of research scientists

do not live with thoughts and hopes of wide recognition and acclaim, and positively revel in such exposure as comes their way. Which is what show business is about.

The acclaim is, of course, somewhat rarefied by the standards of the world. Some may be perfectly happy with the occasional paper in a learned journal, but they are a minority. A paper in *Nature*, a review article in *Scientific American*, these are more attractive as an opportunity to display one's skills to a wider public, even though the wider the public the less discriminating they can be. And the ultimate for most is publicity before a universal and thoroughly (indiscriminating audience) through the mass media.

It is at this level that most scientists become studies in ambiguity. Pleasing as it is to see oneself in print or on television is it not a trifle vulgar, and should the media not be kept at a discreet distance? Surely not. The general public can certainly take a delight in things it does not understand. There are many levels on which one can enjoy a performance of *Hamlet* and there are similarly many levels at which a scientific accomplishment can be savoured. Further, the scientist who refuses to have anything to do with the media is letting in the professional commentator, already at one remove from the field. Misrepresentation is then not the commentator's fault but the scientist's.

Ability to reach a more general public is not at present uniformly spread over the sciences. Although some fields seem to command wide and appreciative audiences, others never seem to project themselves. Chemistry is a case in point. Many chemists entertain serious doubts that their subject can be of interest (other than financial) beyond their own community. This may be true, but it is worth regular efforts to find out whether chemistry has an appeal.

100 years ago



A REUTER's telegram from Aden, of March 23, states that the steamer *Calcutta* arrived there from Zanzibar on the previous day with the body of the late Dr. Livingstone. We fear this must be regarded as final, and as shutting out any further hope; we can only now do all possible honour to those remains which the doctor's faithful servants have so religiously preserved. A letter recently received from Zanzibar, by Mr. R. A. Laing, states that the body, after having been exposed to the sun for a month to dry, and then packed in a hollowed tree, was wrapped round with cloth, and the natives carrying it supposed it a bale of cloth, or kaniki.

From *Nature*, 9, 410, March 26, 1874.

Centre for the practice of theory

Next September, the International Centre for Theoretical Physics in Trieste will celebrate its tenth anniversary. In a decade it has become a major, but still unique, feature of the scientific world. Professor John Ziman, of the University of Bristol, describes its problems and successes.

THE International Centre for Theoretical Physics (ICTP) was inspired by Abdus Salam, professor of theoretical physics at Imperial College, London, who recognised, from personal experience, the need for a place where young and promising research workers from developing countries might come from time to time to "recharge their intellectual batteries". Salam persuaded the International Atomic Energy Agency (IAEA) to create such a centre, which came to Trieste at the invitation of the Italian government and of the City of Trieste.

After some years in temporary premises in the centre of the city, the ICTP moved in 1968 to an elegant building paid for by the regional government and the city. This building overlooks the Adriatic, about 8 km north of Trieste along the main coast road, and has offices and lecture rooms for about 100 physicists at a time. In the summer, naturally enough, the place is crowded, but the building is now in fairly constant use throughout the year. Unfortunately, domestic accommodation has to be found in hotels or lodgings in the city, which means a lot of bus travel; there are long term plans to provide a hostel nearby. The centre has its own excellent library, but the nearest computer facilities are in the University of Trieste. The material facilities are better designed for high-minded individual cerebration than for team research on dirty facts.

Noble laureates and brash young physicists

About 5,000 physicists from 90 different countries have visited the ICTP since it was founded. Of these, at least 1,000 have worked there for periods of several months or more. Visitors range from academicians and Nobel laureates, on courtesy calls of a few days or weeks, to brash young theoretical physicists from developing countries on nine-month fellowships or associateships. But the centre is not an 'institute' with a permanent academic staff committed to a long term research and teaching programme. By arrangement with Imperial College, Professor Salam, the director, spends two-thirds of his time at Trieste, and Paolo Budini, the deputy director, is also a professor at the University of Trieste. Other Trieste professors work regularly at the centre, but do not dominate its activities. In principle the scientific programme of the ICTP is guided by a Scientific Council, nominated by the various sponsoring agencies, which meets for a couple of days annually; in practice, the initiative rests almost entirely with Salam and Budini, in cooperation with various senior scientists who have become involved in the programme of the centre over the years.

A small, hard working technical staff provides administrative, secretarial, library and printing services.

The precise constitution of the ICTP as an international organisation is not easy to explain. Legally it is a part of the IAEA and UNESCO, although the administration is mainly done by the IAEA. Financial support is shared in about equal proportions by the IAEA, UNESCO and the Italian government. Some matters have to be referred to the headquarters of the IAEA at Vienna and to UNESCO in Paris: others are dealt with by a 'consortium' of local Italian academics and officials. Funds for major activities at the centre also come from the United Nations Development Programme (UNDP), the Swedish International Development Agency (SIDA) and, until recently, from the Ford Foundation. Various overt or hidden subsidies come from such bodies as national scientific agencies. An income of about \$1 million a year looks healthy but policy changes, earmarking, exchange fluctuations and other sources of variance or constraint make each year's budget a work of art. The best that can be said of this confused situation is that the centre itself is not burdened with detailed external controls or a heavy bureaucracy, and has been free to find an ecological niche for itself in the jungle of international organisations—who themselves have shown wisdom and restraint in not crushing this strange little beast underfoot.

Changes from experience

Originally, the main purpose of the centre was to provide shelter and intellectual companionship for a number of 'associates'—theoretical physicists of proven competence, taking temporary leave from their academic or government jobs in developing countries for a few months each year to undertake advanced research and to refresh their minds by contact with their peers. This research was to be directed and inspired by distinguished visiting scientists from more advanced nations, for example American professors on sabbatical leave. Naturally enough, opportunities for advanced seminars, small conferences and short study courses would also be exploited as the occasion arose.

It must be admitted that this initial plan has been overshadowed and modified with experience. The quality of the research produced by associates at the centre was very uneven and their need for very close personal supervision could not always be met, simply because there were not enough senior people who could come to Trieste for long periods without financial support. Many of those chosen as associates were ill prepared for self-winding research in an atmosphere of scholarly independence. This defect is difficult to avoid when candidates must be selected on the basis of written applications from a distance and is particularly serious with applicants from small countries with very weak scientific traditions. In some fields of physics, therefore, the associate scheme is now being used mainly to help research workers whose ability has been confirmed by the part they have already taken in other ICTP activities.

Contribution

The research output of the centre (about 100 papers a year, of which half are by physicists from developing countries) is, in fact, quite substantial. In certain specialised fields of elementary particle theory, such as group theoretical methods, the use of non-compact groups and the connections with the theory of gravitation, it is the leading place in the world. In other fields the preprints circulated from the centre often represent important preliminary stages in investigations that are continued and completed elsewhere.

But the contribution of the ICTP to physics should not

be judged from the research actually done there. In recent years, much more emphasis has been given to 'extended seminars', 'winter colleges' and so on, where whole fields of advanced physics are systematically expounded by invited lecturers of the highest international reputation. For each such course, scholarships are given for 30-50 participants from developing countries to come to Trieste for 2-3 months. These courses are open, without fee, to appropriately qualified scientists from all countries and are well patronised by physicists from West Europe. Trieste is geographically well placed to receive physicists from East European countries, whose participation in the ICTP would be even greater if it were not severely limited by exchange controls and other administrative restrictions on travel. The Soviet contribution to the scientific programme has not been as great as was originally hoped but there have been long periods of collaboration at the centre between teams of plasma physicists from the Soviet Union and the United States.



A group of physicists at the ICTP, Trieste.

Many of the best physicists in the world, senior and junior, may be found talking together in the seminars, 'congressini', and 'research workshops' that arise spontaneously during or after each extended course. The centre has thus become a major forum, or market place for the international physics community. Personal contacts are established, ideas are exchanged and new developments communicated in a more leisurely and relaxed environment than the conventional scientific conference or summer school. The general atmosphere is serious and business-like; discussion ranges from the technical problems of mathematical physics to the role of the scientist in a developing country. The typical participant is about 30, has his PhD and several published papers to his credit, and is already carrying responsibility as a university teacher or in a newly formed research group in his own country. But the name on the office door speaks of Nigeria or Brazil, of Pakistan or Korea, rather than the United States, Europe, the Soviet Union or Japan. This is one of the few international scientific institutions in the world that is not dominated by people from economically advanced nations—and that is not just a political talking shop.

In founding the centre, Salam naturally concentrated on his own field of research, the theory of elementary particles. This remains a major component in a scientific programme which now includes regular activities on the physics of nuclei, atoms, molecules, plasmas, condensed matter, living organisms and the Universe, and on mathematical topics

such as computational methods, global analysis, and fluid dynamics.

Highbrow theoretical meetings such as an International Symposium on Contemporary Physics are balanced by courses that get down to hard experimental facts, such as the 1974 Winter College on 'Surface Science'. The centre now caters for the experimental physicist who wants a simple explanation of the theoretical background of his research as well as for the 'theoretician' with his abstract mathematical models. The proportions of the mix are mainly determined by the relative enthusiasms of various individuals who are willing to organise various activities, but would not perhaps be very different if there were a long term plan, based on firm financial guarantees, thought out carefully by a representative committee.

The facilities and programme of the ICTP are now much valued by the world physics community. It is well known as a good place to work at for short periods and the high standard of the extended seminars is coming to be appreciated both by potential lecturers and by their audience. But it still suffers from a shortage of active personal support and practical commitment over long periods from those leading physicists who could help so much in the scientific direction of its activities. Labour is an essential component of the fine ideal of international scientific collaboration.

Serving interests

Does this programme serve the genuine interests of the developing countries? The argument that the real need is for engineers, doctors, technicians and other practical experts is valid on the whole, but ignores the contribution that quite small numbers of well informed and competent academic research workers can make to the solution of technical problems, to educational standards and to cultural life.

Over the years, the activities of the centre have slowly shifted towards the more applicable branches of physics, in response to the political demand for greater 'relevance' to social needs. Indeed, this shift has not been altogether passive: physicists from developing countries meeting together in Trieste as a distinctive group begin to realise for themselves that the pursuit of pure science should not be regarded as an end to itself. In the extended seminar programmes, the intellectual continuity is emphasised between, say, the theory of elementary excitations in covalent solids and the technology of semiconductor devices. This provides a bridge over which the young scientist may move from the academicism of the old-fashioned physics curriculum to the more practical territories of engineering and industry.

A natural extension of the programme of the centre is to support similar activities in other regions of the world. This can only be done on a very limited scale, without permanent facilities, but should eventually strengthen the social and intellectual links established between physicists of various countries when they meet at Trieste. The ICTP cannot, of course, replace the great centres of physics research that exist in advanced countries and that are growing up in many developing countries, and can only supplement the specialist conferences, travel scholarships, regional summer schools and so on that already bind together the world scientific community. But because it is a place where the scientist from a developing country feels he comes as of right, and where people work hard together, on an equal footing, to the highest intellectual standards, it makes a unique contribution to international cooperation and national development. It thus represents a sound prototype for similar institutions in other fields of science and scholarship.

international news

The milk of human unkindness

John Hall

WAR on Want, a British agency pledged to make an urgent political issue of poverty, has accused western manufacturers of causing disease and malnutrition by promoting sales of powdered milks in Third World countries where their use is both unnecessary and unsuitable. In a report called 'The Baby Killer' War on Want says that babies in developing countries are dying because of bottle feeding, and many that do not die are drawn into "a vicious cycle of malnutrition and disease that will leave them physically and intellectually stunted for life".

The basis of the accusation is that sophisticated western marketing methods are being used to persuade mothers in Africa, Asia and Latin America to abandon breast feeding in favour of artificial substitutes. While these substitutes might make perfectly adequate infant foods under proper hygienic conditions, and play an important role in disaster situations when there is no alternative, they become dangerous when used continuously by mothers incapable of reading instructions and in kitchens where there is no clean water for mixing and sterilising.

According to the report the baby food industry stands accused of promoting its products in countries which can't use them properly, and of using advertising and supplying samples and free gift gimmicks that persuade mothers to give up breast feeding, often with fatal results for their children. The mothers often receive the impression that information they receive from commercially based advisers is something in the nature of nutrition education, and 'The Baby Killer' singles out as particularly unethical the use of medically unqualified sales girls, dressed in nurses' uniforms and offering a sales pitch dressed up to look like objective feeding advice.

Other practices which the report describes as unethical and immoral include the encouragement given to mothers to bottle feed while they are still breast feeding satisfactorily, and before there is any need for supplements and the payment of qualified nurses on a sales-related basis belying their educational



role. According to a source within the industry, these nurses are not only paid a commission on sales, but are given an additional incentive in the form of a promise that they will be fired if they don't meet their targets.

The problem of bottle-carried infection is well known in the Third World, if not widely publicised in the west. Even in Britain, where standards of living are comparably high, and most families live in hygienic conditions, hospital statistics show that babies still suffer from infections passed on from feeding bottles. War on Want points out that in the slums of Latin America and the squatter suburbs of Africa, where comparable conditions are hopelessly beyond reach, the choice of an artificial substitute for breast milk is in reality a choice between health and disease—disease caused by an infection or by long term malnutrition.

Breast milk cannot be improved upon as far as infant feeding in underdeveloped countries is concerned: it comes ready mixed, needs no warming or sterilising, leaves no dirty pots and bottles to be washed, and is almost always on tap from its carefully evolved, unbreakable containers. What's more, it contains an appropriate mix of proteins and fats, and seems to offer young infants protection against disease. In Chile, where 20 years ago 95% of one-year-olds were breast fed, the figure is now down to 20% at two months old: at the same time researchers have found that Chilean babies who were bottle fed during the

first three months of their life suffered a mortality rate treble that of breast fed infants.

The Protein Advisory Group of the United Nations (PAG) has produced a table expressing as a percentage of the national minimum wage the cost of artificially feeding a baby. It shows that in Nigeria the cost of feeding a three-month-old infant is about 30% of the minimum urban wage, and by the time the child is six months old the cost will have risen to a crippling 47%. In Pakistan, comparable figures are 40% of the wage at three months, 62% at six months. Not surprisingly, mothers attempting to bottle feed their children at this rate of exchange try to eke out their supplies by overdilution, so that the children to whom the over-watered milk is fed receive an inadequate intake of nourishment. They are left weak, vulnerable to infection, and often end their lives falling prey to wasting diseases like kwashiorkor.

The PAG manual on feeding infants and young children recognises the problem when dealing with powdered milk supplies. "In the less technically developed areas of the world", it says, "immediate and serious basic difficulties attend attempts to artificially feed young infants on a cows' milk formula. These include lack of sufficient money to buy adequate quantities, poor home hygiene (including water supply, fuel, feeding utensils, storage etc) and inadequate nutritional knowledge of the mother. Under these conditions, usual for the

majority in less developed countries, artificial feeds mean the use of too diluted, highly contaminated solutions of cows' milk, resulting at the best in undernutrition, at worst in marasmus and diarrhoeal disease".

And Dr James Farquhar, a reader at the University of Edinburgh and consultant paediatrician at the Royal Infirmary of Edinburgh, reinforces the point in terms which virtually precis War on Want's message: "It is clear to all but those who will not see", says Dr Farquhar, "that informed, adequate and relatively safe bottle feeding must follow, or at least accompany but never precede, literacy, education, infection-free water supplies, sanitation and a standard of living which permits the purchase of enough baby foods, equipment and the means of sterilisation".

The priorities for dealing with the trend away from breast feeding, described by the report as a trend which can only have calamitous consequences, have been stated by the PAG in the following terms:

"The major overall need is to alert governments, health services, nutritionists and the food industry to the emergency situation likely to develop in urban areas in the near future. Its implications are not only the certainty of rising mortalities from almost epidemic marasmus and diarrhoea but also the economic burden of curative services and of obtaining breast milk substitutes on a large scale as well as the long term consequences of the effects of recovered cases of infantile malnutrition on the intellectual level of the community".

War on Want says the aim of its 'Baby Killer' report is to achieve a solution to the problem by opening up the subject to public debate, and it makes recommendations both to the industries and the governments involved. The companies producing dried milk should follow the Swedish example, they say, and refrain from all consumer promotion of breast milk substitutes in high risk communities. They should cooperate constructively with the international organisations working on the problems of infant and child nutrition in the developing countries, and they should abandon promotions to the medical profession which may perform the miseducational function of suggesting that particular brands of milk can overcome the problems of misuse.

Governments of developing countries are recommended to take note of the PAG's advice on national nutrition strategies: and they are advised, where social and economic conditions are such that proprietary infant foods can make little useful contribution, that serious consideration should be given to ending their importation, distribution, and/or promotion.

Astronomers meet administrators

Edward Phillips

THE discussion meeting on astronomy policy arranged by the Royal Astronomical Society in conjunction with the Science Research Council (SRC) turned out to be a staid affair than might have been anticipated, probably because the most vociferous critics of British astronomy are working outside the United Kingdom. There were no outbursts à la Burbidge or Evans. Nor were Sir Fred Hoyle, who two years ago seemed to have fallen foul of British astronomy politics, or Professor Ray Lyttleton, who has been a critic of astronomy policies, at the meeting.

And with the meeting being held in the afternoon of Friday March 1, when the final election results were still coming in, the gathering was mindful of pre-election prophecies that a no-result election would be bad for the economy and therefore bad for big science. The main SRC representatives, Sir Eric Eastwood (Chairman of the Astronomy, Space and Radio Division) and Dr H. Atkinson (Head of the Astronomy, Space and Radio Division) made furtive glances at a blackboard showing the latest election figures whenever anyone mentioned expensive projects.

The election notwithstanding, Dr Atkinson was hopeful that the budget for the Astronomy, Space and Radio Board during the coming financial year will be about £20 million (something less than the cost of making one Concorde), divided up as follows: £4.7 million as the subscription to the European Space Research Organization (ESRO), £2.2 million for minor grants, £2.8 million for SRC establishments such as the Appleton Laboratory and £10.3 million for major facilities such as the projected Mark VA radio telescope for Jodrell Bank, the Northern Hemisphere Observatory (still no site fixed), an infrared flux collector, the Isaac Newton and Anglo-Australian telescopes, and various satellites, rockets and balloons.

The main news to report was that the two crucial committees of the board, the Astronomy Policy and Grants Committee and the Space Policy and Grants Committee, are soon to be folded up. Instead of these two committees which divide the board's parish on the basis of the technique used—astronomy that makes use of satellites would come under the space committee, for example—the SRC is to establish three committees whose interests are defined in scientific terms. An Astronomy I committee will concern itself with radio, X-ray and γ -ray astronomy and with cosmic rays, high energy astronomy in other words. Astro-

nomy, including optical, infrared and ultraviolet astronomy. A Solar System committee will be interested in the Sun, Moon and planets, in solar-terrestrial relations, the Earth's ionosphere and so on. Astronomers will be waiting to see whether new arrangements prevent the perennial research council disease of grant applications falling between two committees.

The meeting also heard that some decision on the much-discussed Northern Hemisphere Observatory is expected this autumn. Professor D. W. N. Stibbs, Chairman of the Astronomy Policy and Grants Committee, said that the SRC is thinking in terms of having three telescopes at the observatory, with apertures of $1\frac{1}{2}$, $2\frac{1}{2}$ and $4\frac{1}{2}$ metres, the largest one possibly having an altazimuth mounting. With Tenerife now

Musical chairs again?

JUDGING from recent advertisements, the British astronomical community is once more to be thrown into turmoil. Four posts are available at the University of Sussex from October 1974, an unspecified number of "positions" at the Institute of Astronomy in Cambridge from the same date, and Professor H. Brück's retirement will, a year later, result in the appointment of a new Astronomer Royal for Scotland and Regius Professor of Astronomy in the University of Edinburgh. No doubt astronomical administrators must also have it in mind that the caretaker Director of the Royal Greenwich Observatory (RGO) also reaches retiring age in the not too distant future.

In recent years, there has been a remarkable shuffling of astronomers around Britain, in some cases back and forth between the same universities; a possible conclusion to be drawn from these musical chairs is that there is a lack of talent at the highest levels. It is certainly difficult to think of two people both acceptable to the establishment and willing and able to handle the jobs at the RGO and Edinburgh. It might be worth reflecting on the possibility that the insecurity engendered by the appointment of researchers for "one, two or three years", and the resulting shuffling sideways at frequent intervals, could be one contributory factor in discouraging the development of sufficient talent both able to do the top jobs and willing to stay in Britain.

apparently out of the running for political reasons, the choice of site seems to be settling down to either Hawaii or Madeira, the Atlantic Island belonging to Portugal. Professor Merle Walker of Lick Observatory, California is acting as a consultant for the SRC during the selection process.

The decision between the two sites seems to be finely balanced. Seeing conditions are generally accepted as better on Hawaii, but by how much better is uncertain, and with the cost of an observatory on Hawaii being higher than Madeira it may yet turn out that, photon for photon, Madeira gives better value.

In the past the Science Research Council has had a reputation for keeping its ideas to itself until much too late in the day. The holding of this special meeting was therefore held to be a step in the right direction, even though it was noted that in the case of the reorganisation of the committee structure the decision had already been taken. Some participants, notably Professor Tom Kaiser (University of Sheffield) wanted even more disclosure of the way the SRC goes about its business and Professor Martin Rees suggested that there be a series of smaller follow-up meetings to discuss particular aspects of the SRC programme.

Water storage in the Wash

Eleanor Lawrence

SINCE 1965 the Water Resources Board has been investigating the possibility of freshwater storage in the Wash estuary on the east coast of England. The board has recently published the interim report on the feasibility study for a proposed scheme costing £140 million, which, if implemented, will eventually provide a major new source of water for the heavily populated south-east corner of England.

A desk study completed in 1970 decided that at present a full barrage enclosing the Wash was technically impossible but authorised a practical study of the first stage of a scheme in which freshwater from the rivers Great Ouse, Nene, Welland and Witham, which flow into the Wash, would be pumped into a system of three or four artificial reservoirs built just offshore. This feasibility study, which includes an ecological survey and the construction of trial embankments, is expected to cost £2.6 million over about six years.

The area has now been completely mapped by aerial, hydrographic and land surveys. And three sets of models are under construction at the Hydraulics Research Station at Wallingford to

study conditions likely to arise from building structures in the Wash and taking water from the inflowing rivers. Geological and geophysical surveys are now complete and have not revealed any major problems.

A trial inshore bank (the bank bounding the landward side of the reservoir) was successfully completed in 1972 and showed that it would be possible to construct the banks using hydraulic fill piped from the existing foreshore sediments. This bank was built entirely from the land and it was intended to construct a trial offshore bank during 1973. In the event no suitable tender was received and so construction was postponed. In view of the present financial position, however, this proposal is being completely re-examined by the government and may be cancelled, especially in view of the board's recommendation that the Wash scheme need not be considered for development until the late 1990s.

Apart from this, the other major outstanding item is the programme of hydraulic model tests. This should be completed by 1975, says the board, and it should then be possible to pronounce on the feasibility of the first stage of the scheme, a reservoir covering 1,700 acres near the mouth of the Great Ouse.

Ecological surveys coordinated by the Natural Environment Research Council have been continuing since 1971. The Wash covers 232 square miles and contains some of the finest salt marsh and mud flat habitat in Britain. The Ouse Washes which are most likely to be affected by the development have been designated by the Nature Conservancy Council as of grade I scientific interest and are included in a register prepared by the International Union for the Conservation of Nature as being of outstanding significance.

The Wash itself is one of the most important breeding and feeding grounds in Britain of the Common Seal which, despite its name, is not so common now. As their main feeding grounds are in the outer Wash, seals are not likely to be affected by development, at least in its initial stages, unless salinity changes affect the type and abundance of food species.

The surrounding mud flats are a particularly important feeding ground for overwintering waders and wildfowl. Monthly counts suggest that the number of waders centred on the area can reach 300,000. One of the four proposed sites for the first reservoir, Bulldog Sands to the east of the mouth of the Great Ouse, is one of the largest feeding grounds for the wading birds whereas another, on the west of the river mouth would be less acceptable to the shellfish industry, which produces about £250,000 worth of various shellfish from

the Wash each year.

Final conclusions on the feasibility and siting of the reservoirs must await the results of the hydraulic model tests, final costings and the possible construction of the offshore bank. The report promises that ecological considerations will be taken into account and the Nature Conservancy Council plans to publish a separate report on overall conservation when the final results of the engineering studies are known.

Physics for physicists

John Hall



PROFESSOR Frederick Guthrie founded the Physical Society in 1874 "for showing new physical facts and new means for showing old ones, for making known new home and foreign discoveries", and one of his regular teaching aids was a compartmentalised box containing specimens of the actual insects, minerals or materials which were the subjects of his lessons. The point was that any number of descriptive talks on, say, cotton spinning, fell short of the mark if a pupil simply had no clue about the appearance, the textures, indeed the nature of raw cotton. These object boxes (origin of the term 'an object lesson') are among the early exhibits in a display of physics memorabilia forming the exhibition "Phonographs to Holographs" at the Science Museum, South Kensington, in honour of the Physical Society's centenary. (Incidentally, one of the historic object boxes pictured contains, under the heading "butterflies", a creature which bears a remarkable resemblance to a Cinnabar moth; but the spirit of the thing was clearly progressive.)

The exhibition runs to an engaging range of Victorian laboratory machines, resplendent in brass and mahogany, and

throws on a number of distinguished investigators sidelights which never made the pages of a text book; Sir William Thomson, for example, is characterised not only as a designer of precision electrical instruments but also as the inventor of a patent water tap. What's more, a good many of the old masters' experiments are reproduced as working models, in the museum's push-button tradition, though the precise value of the demonstrated principle is not always made clear. Kelvin's water dropper, for example, is a pleasant enough looking contraption which intermittently interrupts two streams of water, sends out a spray of droplets and emits a blue spark. An attached rubric explains that this electrostatic induction machine had no practical application, although it led to the design of the Replenisher and the Mouse Mill Generator. Which is all very well if you are acquainted with mouse mill generation, and know its significance (or otherwise).

In fact, as soon as the Victoriana has been digested, the exhibition is plainly a display for the specialist, and calculated to suffuse the museum's staple school-boy customer with indifference. The explanation of the Josephson effect might well have been written in a foreign language and when a layman looks at a superconducting solenoid it does not really help to know that it has a field of 5.4 tesla with a current of 2,300 A. It does not even help to be told that you are looking at a superconducting solenoid. Similarly, two historic pieces of apparatus from the Cavendish Laboratory fall on stony ground if you have not the first idea of what bombardment means, or why it is nice to see the paths of atoms traced in a cloud chamber. C. T. R. Wilson's cloud chamber, described by Rutherford as "the most original and wonderful instrument in scientific history" does not begin to look as though it deserves such a testimonial, and neither does the written explanation supply a justification. A group of youngsters perusing the show on opening day gave about five seconds each to telephones through which one could hear the voices of Wilson and Rutherford describing their work. Five seconds was really not long enough to catch their drift but it was just about long enough to establish that you did not really understand what they were talking about.

So, if you are a physicist, this is an exhibition which you will discover to be packed with nostalgic gadgetry (*pace* Wilson and his cloud chamber) and if you are not a physicist it is an exhibition which tells a tale of the sound and the fury (disintegrating the nucleus of an atom surely qualifies for that description), but may not signify too much. The title of the show springs

from the fact that during the first decade of its existence the most striking foreign discovery that the Physical Society saw was Edison's phonograph; a highlight in recent times was a demonstration of Gabor's Nobel prize-winning holograph. And there at the entrance to the exhibition sits a holograph of Gabor himself, looking for all the world like a Tussauds version of the multiple murderer Reginald Christie. Two more exhibits which deserve mention as eye-catching and reasonably self-explanatory spectacles are a scanning electron microscope (the Cambridge Stereoscan 600, whose blown-up pictures would have looked all that much better in colour) and a pair of motorised mirrors which track back and forth on rails in an experiment measuring the velocity of light. On the whole, this exhibition supplements Rutherford's definition of all science being either physics or stamp collecting. It is also semantics.

First Australian-Soviet science agreement

Peter Pockley, Sydney

THE first agreement between Australia and the Soviet Union for cooperation on science and technology was initiated in Canberra at the end of February. At this stage the formal agreement has been expressed only in the most general terms.

A delegation of three Soviet science administrators paid a preliminary visit to Australia to discuss possible projects of cooperation. The Soviet delegation was at a high level—representing the State Committee, USSR Council of Ministers for Science and Technology were Mr L. N. Yefremov (First Deputy Chairman) and Mr V. A. Kuzin (Head of the Department of Foreign Relations); representing the USSR Academy of Sciences was Mr S. G. Korneev (Chief of the Directorate of Foreign Relations).

The agreement is seen as a quiet first, but significant, step in the Australian Labor government's diplomatic moves to equalise, as far as possible its formal relations between the western, eastern and Asian blocs. Australia has had a binational agreement with the United States for scientific and technical co-operation since 1968. Australian science also has strong links with Britain through the Anglo-Australian Telescope Board and a cooperative programme with Japan for its geostationary meteorological satellite is being evaluated. Soviet and Australian scientists will now work towards the signing of a detailed agreement in Moscow later this year. The Australian Department of Foreign Affairs has recently appointed its first

Senior Adviser on Science, Technology and the Environment, Mr Guy Gresford, an Australian who was formerly Director of Science and Technology at the United Nations.

Linear motors and the EEC

John Wilson

EIGHT manufacturers of linear motors met recently in Brussels, at the request of the Commission of the European Communities, to work out joint proposals for the development of a high power motor suitable for a fast European inter-city transport system.

The companies, representing France, West Germany and the United Kingdom, outlined suggestions for future research but also indicated that the commission must identify the objectives of such a programme more precisely before close collaboration can begin. It is now up to the commission to assess the opinion of the various governments and transport authorities concerned and produce some formal proposals with which the manufacturers can come to grips.

The commission feels strongly that any inter-city transport system for the 1980s, which would involve travel at speeds $>300 \text{ km h}^{-1}$, should be planned on a European basis. Whatever means are used to support the vehicle—wheels, jets of air or magnetic suspension—a high power linear motor is the obvious choice for the traction unit as it overcomes the mechanical difficulties of transmitting sufficient force to the track.

The 'club' of manufacturing companies, including Tracked Hovercraft, General Electric Traction and Linear Motors, first met in June last year and expressed their readiness to embark on a cooperative venture. According to the commission, a good deal of quiet consultation took place afterwards, and that formed the basis of the recent proposals. These are that three cross-company research teams should study the synchronous, asynchronous and transverse-flux motors, respectively; another should examine the problems of collecting electric current at high speed; and a fifth should investigate control systems for the motor.

At first there would be some parallel development of all the motors until a particular type or types were chosen for construction. Then these would be built and tested to evaluate the design.

But these proposals represent a very cautious stage in the negotiations between the members of the club because, although the commission tentatively estimates that it will spend about £2

million on the research programme, over the next two years, no accurate costing has yet been attempted. The companies plan to contribute to the construction and other costs as the commercial stage of the project draws nearer but they hope that the commission will foot the entire bill until it is decided which type of motor will be developed.

There is also some concern that the various governments can reach agreement on the sort of system they want

as readily as the commission seems to suppose. There is little point in the United Kingdom taking part, for example, unless the channel tunnel is in operation. Most population centres in Britain are so close together that, after the introduction of British Rail's new Advanced Passenger Train, journey times are unlikely to be significantly improved.

Unlike an existing rail system, where an individual company can take considerable initiative in its improvement,

a new high speed network requires an enormous capital investment (in civil engineering costs alone) which places it firmly on a national, if not international, level. It seems surprising, therefore, that the commission has waited until now to begin consulting the potential users of the scheme. Without an agreed specification from the governments and others concerned, the technical collaboration of the companies, such as it is, will remain in a vacuum.

correspondence

Oops

SIR,—May we look forward to your tectonics correspondent's report on Aberdeen's 20 km move SSW?¹

Yours faithfully,
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¹ *Nature*, 247, 416 (1974).

Population and politics

SIR,—The article "Is Population a Ministerial Responsibility?" is certainly a thought-provoking one, and makes the main point extremely clearly but it contains a statement which I think should not be allowed to stand unquestioned—that is, "the current demographic position in Britain is that no serious economic or social problems which could be directly attributed to either its population size or growth rate are likely to emerge in the next 30 or 40 years". Further on Mr Cope points out that the Population Panel had argued the necessity of bringing home to the public the need to consider the aggregate consequences of their family building behaviour, in spite of the possible conflict of these consequences with individual preferences.

One point which immediately springs to mind is that, notwithstanding the very considerable publicity that has been given to the existence of family income supplements and similar benefits of this type, a large proportion of the group for whom they are meant are unaware of the existence of the benefits to which they are entitled, yet the limited evidence available in the report of the Population Panel suggests that this particular group, who form most of socio-economic group 5, are con-

sistently among those with the highest reproduction rate. Although there is clearly scope for a great deal of work in fields relating the family's economic status to family size as matters stand (and with the example of one difficulty in communication of a real need being all too apparent) it seems to me highly premature—if not completely wrong—to suggest that continuation of trends such as this could not give rise to serious social problems over the next 30 or 40 years. An equal problem may arise by the over-reaction to publicity of groups such as socio-economic group 1.

Yours faithfully,
P. J. HEALD

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Cigarettes and guilt

SIR,—Although any contribution to the problem of deterring cigarette smoking by pregnant women is welcome, the airing given to it in your leading article of February 15 seems to me to need correction.

Your suggestion that a different approach would have been more effective and humane is not one which the careful preparatory work which we did bears out. A substantial survey of the reaction of pregnant women to the approach adopted, and a smaller one concerning the use of the naked figure, established quite clearly that the message and its form were entirely acceptable to those for whom it is intended. Conversely, much experience of the use, mainly by other organisations, of soft and pretty material has established that it is, as regards topics as serious and difficult as this one, largely ineffective. In-

cidentally, the effort we are making represents not only a new dimension in a continuing campaign against smoking in general; it is a specific effort to tackle the most clearly avoidable of all the factors affecting perinatal mortality.

The "well informed doctor and nurse" to whom you refer are a vital element in the campaign. Almost all the general medical practitioners and midwives in practice, and a great many clinic staffs, have received a personal letter setting out the scientific case for the campaign and inviting participation in exactly the way you have in mind, using our leaflet if they so wish.

Finally, of course we agree with your suggestion that information should be given to school girls about this topic and, indeed, several others affecting their health and well being. But this is a different exercise to which we are addressing ourselves as a separate activity.

Yours faithfully,
A. C. L. MACKIE

The Health Education Council,
78 New Oxford Street,
London WC1A 1AH, UK

Survival of science

SIR,—And, in an editorial¹, grovelling low
Even at the base of Maddox' statua
Which all the while ran blood, great
Nature fell,
O, what a fall was there, my country-
men!
Then I, and you, and all of us fell down,
Whilst bloody doomsday flourish'd over
us.

Yours faithfully,
THOMAS H. JUKES

Berkeley, California

¹ *Nature*, 246, 439 (1973).

news and views

Fermi surface of magnetic rare earths

THREE physicists at the University of Birmingham have reported direct measurements of the Fermi surface of gadolinium metal (Young, Jordan and Jones, *Phys. Rev. Lett.*, **31**, 1473; 1973). This is the first experimental observation of the Fermi surface of a magnetic rare earth element, although there have been some preliminary measurements reported by workers at the Ames Laboratory in the United States on a sample of non-magnetic lutetium (Hoekstra and Phillips, *Phys. Rev.*, **B4**, 4184; 1971).

In a metal some of the electrons are lost by the atoms to the conduction band. If these electrons were perfectly free they would have a quasicontinuous range of allowed energies up to a maximum energy, the Fermi energy. The electrons move through the periodic lattice of the positive ions, however, and these cause the formation of allowed and forbidden energy bands. In anisotropic three-dimensional materials the energy bands are also anisotropic and the Fermi surface, the energy surface formed by the Fermi energy in three-dimensional reciprocal space, also becomes non-spherical. These concepts of band structure and Fermi surface are vital in understanding the magnetic, electrical and thermal properties of metals.

There have been several very careful calculations of the band structure (Keeton and Loucks, *Phys. Rev.*, **168**, 672; 1968; Jackson, *Phys. Rev.*, **178**, 949; 1969; Dimmock and Freeman, *Phys. Rev. Lett.*, **13**, 750; 1964), and hence the Fermi surface, of the rare earth metals. In particular the magnetic properties have been interpreted in terms of very small variations of apparently insignificant parts of the theoretical Fermi surface (Evenson and Liu, *Phys. Rev.*, **178**, 783; 1969). Obviously before more detailed theoretical calculations are started there has been a need for some supporting experimental evidence.

Problems have arisen, however, because techniques which allow measurements of the Fermi surface require very pure single crystal materials. It is only in these samples that the electrons can traverse reasonable distances of the Fermi surface without suffering scattering. The rare earths have proved difficult to purify for a variety of reasons. The elements in the series all have identical outer electronic structure and hence chemical separation is difficult. Relatively pure samples of rare earth oxide can best be obtained by ion-exchange techniques and the metal produced from the oxide by a conversion to the fluoride followed by a calcium reduction of the fluoride. Added to this the individual elements are highly reactive, readily forming nitrides, hydrides, oxides and carbides both during preparation and on subsequent exposure to the atmosphere. Until very recently samples more than 99% pure with respect to all constituents were very difficult to manufacture.

The Birmingham workers have used the technique of solid state electrolysis in ultra-high vacuum (UHV) to improve the purity of their gadolinium samples, particularly with respect to the gaseous and carbon impurities. The initial material is taken in the form of a polycrystalline rod and placed between electrical contacts in a UHV system. A current of 500 A cm⁻² is passed through the sample heating it to 1,100° C. The metallurgy of this process is not well

understood but it is thought that during the electrolysis the oxygen impurities among others migrate to the anode end of the sample, while the hydrogen is simply released in the UHV heating. Hence a section cut from the centre of the sample is found to be purer than the starting material. If this subsample is reprocessed the purity improves still further. An added advantage of this purification process is that preferential grain growth takes place in the centre of the rod, producing single crystals with dimensions of some millimetres.

A simple, though for the rare earths a sometimes unreliable, method of checking the purity of the materials is to measure the resistance ratio (the ratio of the electrical resistance at 300 K to that at 4.2 K). The higher this value the purer the sample. The resistance ratio for these latest Gd samples (240) is an order of magnitude better than for previous material. The Ames lutetium had a resistance ratio of 21.

The de Haas-van Alphen technique, in which on cooling the sample to about 1 K it is found that the magnetisation is periodic with increasing internal magnetic field, has been used to study the Fermi surface of the pure Gd. The frequency of the periodicity can be associated with the frequency of a particular electron orbit around the Fermi surface. Young *et al.* were able to observe four separate de Haas-van Alphen frequencies for a magnetic field applied in the base plane of the hexagonal structure and a further three frequencies for a magnetic field applied along the hexagonal axis.

The higher experimentally observed frequencies can be associated with orbits round the theoretically predicted Fermi surface. For example, the theoretical Fermi surface is basically columnar in shape and oscillations around the trunk of the column can be observed and the diameter of the column estimated. The lower frequencies reported cannot, however, be explained with the theoretical Fermi surface at present established. As happens at regular intervals in developing areas of research, it seems that the tables have been turned. Until this paper was published theoreticians were demanding measurements of the Fermi surface to check their calculations. Now the experimentalists would like more sophisticated theory to explain their results.

Of course, these pure samples of single-crystal rare earth elements will start a new series of measurements on those physical properties that depend on purity. Already Wells, Lanchester and Lee at the University of Southampton have measured the specific heat of Gd at low temperatures and found no evidence for the anomaly at 3–4 K reported by previous workers. This anomaly can now be clearly attributed to Gd₂O₃ impurity present in the previous samples.

S. B. P.

Immunity to measles and canine distemper viruses

THE role of cell-mediated immunity in recovery from virus infection is a subject which is currently receiving considerable attention. It has long been recognised that dysgamma-globulinaemias—persons with deficiencies in their ability to produce humoral antibodies—recover normally from certain virus infections and can be immunised successfully against

smallpox. More recently, the key role of cell-mediated immunity in recovery from some of these diseases has been demonstrated experimentally by inoculating virus into mice whose ability to produce circulating antibody has been destroyed by the injection of cyclophosphamide. It now seems probable that, whereas recovery from enterovirus and arthropod-borne infections is due to the production of humoral antibody, recovery from herpesvirus, poxvirus and, possibly, myxovirus infections is largely a consequence of cell-mediated immunity, though cooperation between T and B lymphocytes has been demonstrated in several systems (see Allison, *Proc. R. Soc. Med.*, **66**, 1151-4; 1973).

The demonstration by Brown and McCarthy of a cell-mediated hypersensitivity reaction to canine distemper virus in dogs sensitised with measles virus (see page 344 of this issue of *Nature*) may throw new light on the nature of the immunity to distemper conferred by the inoculation of measles virus. Puppies born of distemper-immune bitches receive immunoglobulins in the colostrum and these not only protect against infection but also prevent successful immunisation with live distemper vaccine. Measles virus is not neutralised by distemper antibody and, provided sufficient is administered, can protect against subsequent challenge with distemper virus. Vaccination of dogs with measles virus may result in the production of some measles antibody but this has no neutralising effect on distemper virus *in vitro* and no distemper antibody is formed. This, together with the fact that protection is related to the dose of measles virus administered, suggests that the heterotypic virus functions in a similar way to the first dose of an inactivated vaccine, serving to stimulate a primary but not a secondary immune response (Prydie, *Vet. Rec.*, **83**, 554-9; 1968). On the other hand, a single dose of an inactivated vaccine does not usually produce a solidly-immune state and there is evidence that the efficacy of the vaccine is destroyed by treatment with formalin. Clearly, the mechanisms of heterotypic resistance cannot be satisfactorily explained on the basis of a humoral antibody response. The possibility that cell-mediated immunity may be the principal immune mechanism involved provides an exciting challenge for future study.

The work of Brown and McCarthy should also serve to stimulate further work on the mechanisms operative in recovery from canine distemper. There is considerable variation in the facility with which individual dogs recover from the disease and several attempts have been made to correlate recovery with the rate at which serum antibody is formed; it has been claimed that unless a neutralising antibody titre of about 1 in 100 (against 100-300 EID₅₀ in the standard *in ovo* neutralisation test), is present by the fourteenth day after infection, the dog is likely to succumb. This view fails to take account of the evidence that dysgammaglobulinaemic children recover uneventfully from measles. Moreover, the neutralisation test measures only those antibody molecules which have not yet complexed with virus antigen; in an animal whose tissues are still actively producing virus, a low neutralising titre does not necessarily imply a deficiency in the humoral antibody response because a proportion of the antibody formed will be complexed with virus. Clearly, much remains to be done before the relative importance of different types of immunity in recovery from virus infection is fully understood.

H.J.C.C.

Where do meteorites come from?

Nobody knows for certain where these large chunks of interplanetary matter come from. The ones that have been picked up from the Earth's surface have mineralogical and crystal-

lographical characteristics which were probably produced during the early stages of planetary formation. These meteorites all seem to be transient members of near space, however, their life times as individual entities, away from their parent body, being several orders of magnitude shorter than the age of the Solar System.

Present theories fall into three main categories. First, meteorites could have an asteroidal origin, being produced directly as fragments of colliding asteroids in the asteroid belt or from those few asteroids which happen to cross the orbit of Mars. Knowledge of asteroidal bodies is very sketchy, but their orbits can be found easily and their diameters can be estimated from the intensity of the reflected light and from very careful direct optical measurements. Masses, densities and internal structure (apart from the measurement of the mass of Vesta by Hertz (*Science*, **160**, 299; 1968)) have all been estimated by assuming asteroids are made of meteoritic material; so one is back to where one started from. Two problems rear their heads with this theory. The short period of exposure to cosmic rays—even if erosion sputtering due to collision with microparticles in the Solar System dust cloud is taken into account—implies a demand on supply that is several orders of magnitude greater than the mass of debris produced by asteroidal collisions. It is also very difficult dynamically to bring a particle from the collision position in the asteroidal belt into the inner Solar System. The mean relative collision velocity of asteroids is around 5 km s⁻¹ and an impulse of this order in the retrograde direction is required to change the orbit into one that intersects the Earth. The Mars asteroids are too few to yield sufficient debris.

Lunar ejecta has also been suggested as a source, but the mean geocentric velocity of this ejecta when captured by the Earth would be very low and also, because of pulverisation, large particles have little chance of survival. Öpik (*Adv. Astron. Astrophys.*, **4**, 301; 1966) calculates that about 0.2% of the meteorites have a lunar origin.

The third hypothesis has meteorites originating in comets. It must be remembered that as meteorites have such a short exposure age, their parent bodies must still exist and should be an observable class of celestial object. Stone and iron meteorites could come from cometary nuclei, the thick layers of icy conglomerate (in the Whipple model of the nucleus) shielding them from cosmic rays for most of their lifetime. The longer exposure ages of iron meteorites could be easily explained by the lower erosion rate of the surface of iron meteorites relative to stones. But there is little evidence of any correlation between meteor showers (which occur when the Earth goes through the toroid of dust debris produced by decaying comets) and the fall of meteorites. This could be due to the large gas pressure required to blow particles away from the gravitational attraction of the nucleus; in other words, large particles of the size of meteorites (> 10⁵ g) might never get away whereas the smaller visual and radio meteors (10⁴ → 10⁻⁵ g) do. Öpik finds that the biggest particles that can escape have diameters less than $17.3q^{-2}d^{-1}$ cm where q is the perihelion distance in astronomical units and d is the diameter of the nucleus of km. Comet Encke could therefore lose metre-sized chunks and large meteoritic fragments have been associated with the Taurid stream which originated from Encke. Of course the cometary origin still leaves the question of how the meteorites got into the nucleus in the first place.

One way of deciding between these theories is to analyse the structure of meteorites in more detail, hoping that additional knowledge might provide the vital clues. Results of this type of detailed analysis have been put forward in three recent papers in *Science*. MacDougall, Rajan and Price of the Department of Physics, Berkeley, University of California have found (183, 73; 1974) that the individual grains and chondrules in gas rich meteorites have high densities of particle tracks and severe track density gradients caused by low energy particle irradiation from nuclei accelerated by

solar flares. This irradiation damage was also non-uniform. They find that track density gradients exist not only on the particle surfaces but also at internal broken edges in the meteorite, indicating that the meteorite compacted more than 10,000 years after the grains were formed. The gradient is also so steep that only a very tenuous atmosphere ($\lesssim 10^{-8} \text{ g cm}^{-3}$) could have intervened between the Sun and the meteorite. The non-uniformity of the irradiated features of the meteorites Kapoeta and Fayetteville are similar to those found in lunar soil. Grains also seem to have been shocked after formation, possibly during meteorite compaction.

Brownlee of the Department of Astronomy, University of Washington and Rajan have found (182, 1341; 1973) micrometeorite craters, caused by hypervelocity impacts ($>4 \text{ km s}^{-1}$), on the chondrule-like spherical objects in the meteorite Kapoeta. These craters are very similar to those found on lunar glasses. By comparing the meteorite with lunar materials, the authors conclude that the micrometeorite flux in the past was about an order of magnitude higher than it is at present, and that the chondrule spheres are droplets of once melted ejecta produced by cratering events on the parent body of Kapoeta. The rarity of these spheres in comparison with their relative abundance in lunar soils indicates that this parent body is of asteroidal size. Brownlee and Rajan conclude that the meteorite Kapoeta was formed by impact processes on an impact-generated regolith, this regolith being on an asteroidal-sized body; and that this process occurred during the latter stages of the accretion mechanism that formed the planetary bodies of the Solar System. It has also been found that the physical properties of interplanetary dust have not changed appreciably over the lifetime of the Solar System even though the flux was higher in the past. This implies a long term constancy of cometary phenomena in the inner Solar System.

Lange and Larimer (Center for Meteorite Studies, Arizona State University) have also been looking at chondrules (Science, 182, 920; 1973), this time in the meteorite Ngawi. They conclude, however, that they were formed by flash heating and remelting of dust grains caused by high velocity ($1\text{--}10 \text{ km s}^{-1}$) impacts in the cooling gas nebula that formed the Solar System.

It seems that chondrules could be formed right at the beginning of the Solar System or on the surface of an asteroid. Also the upper limit to the atmospheric gas pressure at the meteorite surface calculated by MacDougall, Rajan and Price does not exclude a cometary nucleus as the source of meteorites (do not forget that this nucleus is also being bombarded by interplane-

tary particles). So even now all the options are open and the origin of meteorites is still a mystery.

D.W.H.

Activation of latent leukaemia viruses

from a Correspondent

IN 1970 Hirsch, Black, Tracy, Leibowitz and Schwartz (Proc. natn. Acad. Sci., U.S.A., 67, 1914) reported that graft-versus-host (GvH) reactions in BALB/c mice activated leukaemia viruses that otherwise remained latent or repressed. Hirsch and his colleagues then proceeded to demonstrate leukaemia virus activation during homograft reactions provided that immunosuppressive agents were also administered (Science, 180, 500; 1973) and in mixed lymphocyte reactions *in vitro* (Proc. natn. Acad. Sci., U.S.A., 69, 1909; 1972). Since stimulation of lymphocyte proliferation by mitogens such as phytohaemagglutinin did not activate leukaemia viruses, these authors concluded that virus activation requires specific immunological stimulation.

Could the activated virus be responsible for the high frequency of lymphoreticular tumours found in mice undergoing chronic GvH reaction? Armstrong,

Black and Richards (Nature new Biol., 235, 153; 1973) found that ultrafiltrates of spleens from mice undergoing GvH reactions induced leukaemia when inoculated into newborn mice. It is also well known that New Zealand Black (NZB) mice which suffer spontaneous autoimmune disease have a high level of endogenous leukaemia virus activity and a high incidence of lymphoreticular tumours.

It is becoming evident that the leukaemia viruses associated with chronic immune reactions in mice have a peculiar host range. They can neither be classified with the N-tropic nor B-tropic murine leukaemia viruses because mouse cells themselves are resistant to infection whereas certain cells of other species are susceptible. The virus of NZB mice has been studied by Levy and Pincus (Science, 170, 326; 1970) who found that it is non-infectious for mouse cells but infects rat cells. Levy now reports (Science, 182, 1151; 1973) that similar viruses can be isolated from normal spleens and kidneys of NIH-Swiss and C57BL mice. These tissues were co-cultivated with non-producer NRK cells transformed by Harvey strain murine sarcoma virus, whereupon a pseudotype of the sarcoma virus was produced. This pseudotype infected NRK cells and possessed similar biological and immunological properties as the pseudotype obtained with virus from NZB mice. Immunosuppressed NIH-Swiss mice tissues gave rise to the highest titre of pseudotype. Scherr, Lieber and Todaro in the first issue of Cell (1, 55; 1974) report that a virus activated in BALB/c cells *in vitro* during mixed splenocyte reactions does not infect mouse cells but replicates most efficiently in a rabbit cell line. This virus is probably closely related if not identical to the viruses isolated by Levy. Thus a virus can be vertically transmitted in one species but when activated may only be able to infect cells of other species. Levy calls such viruses "xenotropic".

Another example of xenotropism is the endogenous feline virus which when activated replicates in human cells. This virus was originally detected after inoculation of RD cells, derived from a human rhabdomyosarcoma (McAllister *et al.*, Nature, 235, 3; 1972), into foetal cats *in utero* (again, an immunologically undeveloped host) and was at first regarded as a possible human virus (see Nature, 244, 252; 1973). A similar experimental model was used by Todaro *et al.* (Proc. natn. Acad. Sci., U.S.A., 70, 859; 1973) to isolate a virus from NIH Swiss mice which had been immunologically suppressed with antithymocyte serum and inoculated with RD cells. This virus, AT-124, contains group-specific antigen and RNA-dependent DNA polymerase related to murine C-

Vegetable anatomy



At first glance, few readers would associate this figure with Indian hemp, cannabis or *Cannabis sativa*. It shows, however, the anatomy of parts of this plant (for example, the epidermis of a bract) and is included in a new reissue of an atlas of microscopy published "for use in the identification and authentication of some plant materials employed as medicinal agents" (Powdered Vegetable Drugs by Jackson and Snowden; Thornes, London, 1974, £4.00).

type viruses but grows preferentially in cat and primate cells and not in mouse cells. It remains to be elucidated whether this xenotropic murine virus is identical to the immunologically-activated virus. The presence of a transplant from a permissive host in an immunosuppressed recipient provides ideal conditions for the proliferation of xenotropic viruses.

It can hardly have escaped the attention of any of these investigators that human leukaemia viruses, if they exist, might be activated by chronic immunological reactions or by immunosuppression. Such a virus could account for the increased incidence of lymphoreticular tumours found in patients suffering autoimmune disease or carrying organ transplants. Since nobody has yet cried "Eureka", one must assume that mixed human lymphocyte cultures do not activate leukaemia viruses as readily as those of BALB/c mice. This is perhaps not surprising since Hirsch *et al.* found that the immunological activation of murine leukaemia virus could not be observed with all mouse strains. Furthermore, if the activated human virus were

also xenotropic, its discovery may depend on finding the appropriate foreign cell type in which to grow the virus to detectable levels.

Alpha clustering inside nuclei

from our Nuclear Theory Correspondent

MANY different lines of evidence indicate that the nucleons in the nuclear surface tend to group together to form α particles. The cross sections for the elastic scattering of α particles by nuclei and of α -transfer reactions require a significant degree of α clustering. Hartree-Fock calculations of the structure of light nuclei show a pronounced tendency to form groups of four nucleons that can be interpreted as α clusters.

A new approach to the problem of α clustering in nuclei has recently been made by Brink and Castro (*Nucl. Phys. A216*, 109; 1973). They were primarily interested in finding out whether there is any tendency to α clustering in the interior of heavy nuclei, so to simplify their calculations they considered infinite nuclear matter so that all surface and Coulomb effects could be neglected.

Two types of infinite nuclear matter were dealt with: one consisting of nucleons represented by plane waves and the other consisting of groups of four nucleons (α clusters) in a regular crystalline array. If a particular form of the nucleon-nucleon interaction is assumed, the total binding energy of the system may be calculated by standard quantum mechanical procedures. It is then possible to see whether the array of α particles is more stable than the nucleons and this in turn has implications for the structure of finite nuclei.

In such a calculation the results must not depend too critically on the assumptions made, so Brink and Castro used two extreme types of nuclear force (the Brink-Boeker and the Skyrme) and three types of crystalline array (the simple, body-centred and face-centred cubic lattices). In each case it was found that for the nucleon densities corresponding to those in the centre of heavy nuclei the nucleon matter was more stable than the α -cluster matter. This suggests that the α clustering inside heavy nuclei is rather small.

For a series of different nucleon densities, it was found that when the density is reduced to about one-third of that inside heavy nuclei the α -cluster matter becomes more stable. Thus as the density of nuclear matter is reduced there comes a point where it condenses into α particles. This is to be expected from the high binding energy of the α particle and the condensation takes place as soon as the density is low

enough to prevent the interactions with all the nearby nucleons from breaking up any incipient clusters that may be formed.

The actual density at which the condensation occurs naturally depends on the nuclear force and on the form of the crystal lattice assumed in the calculation; but since the ones used were deliberately chosen to be rather extreme, any other realistic force or lattice will give results similar to those already obtained. Thus the general conclusion is not affected by the details of the assumptions made in the calculation.

It is natural to apply these results to the nuclear surface, where the density falls rapidly to zero, and to infer that in the outer region where the density is low some degree of α clustering will occur. This argument is very plausible qualitatively, although the complications of a finite system with a curved surface and the presence of the Coulomb interaction alters the value of the critical condensation density. Nevertheless this work has shown in a simple way that we may expect to find some degree of α clustering in the nuclear surface, but not in the interior.

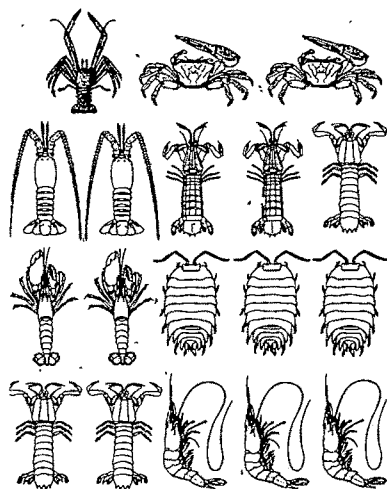
Skeletonising the polypeptide

from a Correspondent

PROTEIN crystallography has progressed so rapidly in recent years, and so many new structures have been solved, that speakers at current meetings in the field generally give little information on how their results are actually obtained, that is, the pure mechanics of the business. They rather prefer to persuade the audience of the specific and general biochemical significance of their latest elaborations, beautiful atomic models in wire or ball-and-stick, photographed and presented stereoscopically. For the more traditional crystallographer in the audience, well aware as he is of the overriding biological aim of protein work, the implication that his métier is, though important, somewhat humdrum and routine, strikes a hollow note.

It is refreshing, therefore, to come across new papers which re-emphasise the enormous difficulties still to be overcome in the analysis of a structure as complex as a protein crystal, and which introduce new techniques for tackling these difficulties. Increasingly, computers have been used, and, if heavy-atom derivatives of the protein can be prepared and suitable automated X-ray diffraction apparatus is at hand, then there are many programs available to prepare the data and calculate the three-dimensional Fourier synthesis of electron

Crustaceorum Catalogus



THE Pinnotheridae (Decapoda, Brachyura)—the crustacean family whose members are commensal or parasitic—are on file in the latest part of the *Crustaceorum Catalogus* (part 3, edit. by Schmitt, McCain and Davidson; Junk, The Hague, 1973, 76 Dutch guilders). The catalogue is basically a compilation of the references contained in the pinnotherid sections of two card files started by Schmitt in the Smithsonian Institution, Washington. All pinnotherid references to 1968 and a few more recent publications are included.

density. The problem that remains, that of assigning a polypeptide chain to the electron density, can involve many months of painstaking model-building work, and has hitherto been relatively unassisted by computer methods.

An important mechanical aid in model construction incorporates a half-silvered mirror, placed between the model and a plot of the electron density on perspex sheets, and arranged at such an angle that the model and the map appear superimposed to the viewer. This simple but ingenious device, called after its inventor, Professor Frederic Richards of Yale University, the Richards' box (by those in the trade, it is known more familiarly and affectionately as 'Fred's folly') has proved an indispensable aid. Even so, much experience and knowledge is required by the crystallographer to unravel and interpret the electron density map, and the prospect of programming a computer to take over this human task is daunting. Nevertheless, Greer (*J. molec. Biol.*, 82, 279; 1974) has presented a method which goes a long way towards the solution of this problem.

Often, the amino acid sequence of the protein is known and this greatly assists the interpretation of the electron density map. The more clearly recognisable residues in the map may be correlated with the sequence and the more obscure ones inferred by extrapolation. To make the situation more general, however, Greer eschews the use of sequence information. Instead, he aims to produce a skeleton outline of the polypeptide chain by progressively chipping away at the density map.

The electron density map, of resolution between 2 and 3 Å, is plotted on a Cartesian grid of spacing about 1 Å. Certain criteria are established for the selection or rejection of points on the grid. A point is removed if its density is in the interval being examined in the current 'pass' through the map, and if no 'hole' is created by its removal. To test for the latter condition, the relative densities of the 26 nearest-neighbour grid points were examined. Further prerequisites for removal are that the point should not be at the 'tip' of a density region (for example, at the end of a side chain) and that the removal should not destroy the continuity of the skeleton (this requires a search of nearest-neighbour relationships).

An illustration of the application of these rules is given for a simulated two-dimensional map representing a polypeptide of thirteen residues. In order to isolate the main chain, the side chains, defined in each case as the skeletal piece between a 'tip' and 'branch point', are removed and their positions flagged. An exception to this procedure has to be made at the end of the main chain,

defined by the coincidence of two side chain branches to a single branch point, since it is not known at this stage which is the main chain and which the terminal side chain. Main chain cross-bridges, due either to disulphide bridges or to hydrogen bonds are recorded.

The next major problem is to isolate a single molecule from its neighbours. The boundary is often poorly defined in the map and there may be considerable interpenetration of adjacent polypeptides. To eliminate unwanted chains, the symmetry operators and unit cell translations of the crystal are applied to each point or group of points to check whether it occurs more than once in the volume defined for one molecule. The redundant points are then chosen as those furthest from the centre of gravity of the molecule. There may still be unwanted pieces of neighbouring molecules present after this procedure, and the program searches for and eliminates all small chains that have both ends more than about 4 Å from any point on the skeleton. To reinstate portions of the main chain that may occasionally be deleted by mistake, the skeletons before and after the above procedures are compared, and the chain is examined for continuity.

The skeleton now represents the main chain punctuated by main chain branch points and side chain markers. These junctions indicate the positions of some of the α -carbon positions, and others

can be found by invoking the so-called 4 Å rule, that the separation of residues along a polypeptide chain is always about 4 Å. Greer is developing methods for further automation of the map interpretation, but meanwhile the existing program already provides a head start for further model-building work by conventional techniques.

The program is applied to the 2.0 Å map of ribonuclease S, for which the structure is well established. A certain amount of manipulation is required to optimise its performance, but the net result is a set of residue positions which strikingly resembles that achieved by conventional methods. The mean deviation of measured and predicted α -carbon positions is 1.37 Å, which indicates good agreement since the skeleton was calculated on a 1 Å grid. The skeleton shows features such as the β -structure regions and the disulphide bridges clearly, but there are some trouble areas. Occasionally, three, four or five-membered rings occur instead of a straight chain, but these features are easy to detect and eliminate. Sometimes small pieces of chain are missing where the density of the map is low.

The comparative layman may easily be misled by the elegance and apparent ease with which computer techniques can produce seemingly convincing structural models of dazzling complexity, and it is necessary to be especially critical of the predictions made. In this paper

Sunspots affect Indian monsoon

EVIDENCE that the solar cycle produces a marked effect on the weather on Earth continues to accumulate. King's recent study (*Nature*, 245, 443; 1973) gathered sufficient evidence that there could remain little doubt of the reality of the effect; a report by Jagannathan and Bhalme emphasises that this effect is a major one, altering the circulation patterns of the terrestrial atmosphere significantly.

This evidence comes from a study of variations in south-west monsoon rainfall in India between 1901 and 1951 (*Mon. Weath. Rev.*, 101, 691; 1973); the polynomial trend analyses and power spectrum analyses used reveal "composite patterns corresponding to the epochs of sunspot maximum, sunspot minimum, sunspot increasing and sunspot decreasing". The authors mention that 1899 was a year of severe famine on the Indian plains, brought about by extreme drought conditions, and that 1917 was a year of

very heavy rainfall. No doubt students of the link between solar cycle and the weather will be intrigued to note that these were years of minimum and maximum solar activity, respectively; although Jagannathan and Bhalme do not specifically mention the present serious droughts in India, it seems difficult to avoid the conclusion that these may therefore be linked to the present period of low solar activity.

That would certainly fit the "composite patterns" noted; it seems particularly unfortunate that the link and these patterns were not discovered sooner, when they might have provided forwarning of the present situation. But it is unlikely that such warnings would have been taken seriously a few years ago; it is to be hoped, however, that research into these climatic variations will be accorded the recognition which the progress now being made justifies.

the firm results are the proposed α -carbon positions, and the correspondence of these to the known positions of ribonuclease S is convincingly demonstrated. It will be exciting to see the method applied to a completely new and unknown polypeptide or protein.

New structure, new mechanism for trypsin

from a Correspondent

It may well be true that protein and enzyme crystallography has passed its zenith. But many laboratories engaged in this activity still continue to produce results that are often interesting, and even important. As the number of structures unravelled continues to increase, particular emphasis is being placed on structure-function relationships as well as on correlations between members of enzyme families.

The serine proteases are all enzymes involved in peptide bond cleavage during the processes of digestion, and the structures of two of them, chymotrypsin and elastase, were determined by X-ray analysis several years ago. Stroud and his colleagues now report the details of their studies on inhibited complexes of a third such enzyme, trypsin. Inhibition of trypsin by diisopropylfluoro-phosphate proceeds irreversibly, to give a complex, DIP-trypsin, and Stroud, Kay and Dickerson (*J. molec. Biol.*, **82**, 185; 1974) have determined its three-dimensional structure, initially at low, and then at higher (2.7Å) resolution. It is notable that the overall folding of the DIP-trypsin molecule is topologically very similar to that previously observed in chymotrypsin and elastase; the overall identity of sequence between these three is only 24%. The active-site architectures are likewise closely related. DIP reacts uniquely with Ser 195 and the position of the γ -oxygen of this residue corresponds closely to that in acylchymotrypsin.

Trypsin is also specifically and reversibly inhibited by benzamidine, in a way that probably mimics arginine side chains in a specific substrate. Krieger, Kay and Stroud have also determined the structure of this complex, which they describe in an accompanying article (*ibid.*, 209). The siting of the benzamidine in the enzyme-binding pocket, has induced alterations in the geometry of this pocket so that, in particular, it has undergone a marked closure so as to 'sandwich' the aromatic ring of the inhibitor molecule in between several peptide planes.

From the comparison at neutral pH of the structures of these two quite different inhibited derivatives of trypsin, Krieger *et al.* attempt to obtain information concerning the mechanism of the

enzyme action. DIP-trypsin can be considered to be a model for the 'tetrahedral' intermediate widely accepted to be a covalent intermediate in the catalysis of serine proteases—whereas the benzamidine-trypsin complex may well represent a Michaelis complex (or indeed any other non-covalent intermediate, such as the often-suggested trans-conformation complex). Benzamidine is, in effect, a powerful competitive inhibitor for specific trypsin substrates, and it is quite reasonable to suppose that it binds in the same way as them. Moreover, the benzamidine-trypsin complex is stable at pH 8, when the enzyme is totally in its active conformation. Any observed change in the protein structure may then be solely attributed to the actual binding of the pseudo-substrate, and not to any shift in the balance between active and inactive conformations. The structure of active free trypsin remains to be solved, although Stroud *et al.* believe that DIP-trypsin should resemble it quite closely.

Insertion of benzamidine into the binding pocket involves the formation of a salt bridge with the β -carboxyl group of Asp 189, this residue accounting for the unique specificity of trypsin among the serine proteases for positively charged substrates. It is striking that this bridge is formed without any movement of the carboxylic acid, this residue being constrained by the tertiary structure. Any other substrate whose basic side chain has not the required length, will then, in forming the salt bridge, move the susceptible bond of the substrate away from its optimal catalysis position. The binding of benzamidine effectively induces certain changes in the active site, which in all favour rapid subsequent hydrolysis in the case of the specific substrates. The readjustment of the position of the most important residues of the enzyme when it binds a positively-charged substrate, may also explain the activating power of small amines for the hydrolysis of non-specific substrates.

It is worth noting that the charge-relay transfer mechanism involving the catalytic triad Asp₁₀₂, His₅₇ and Ser₁₉₅ proposed by Blow *et al.* (*Nature*, **221**, 337; 1969), for the acylation and deacylation of chymotrypsin, can also be applied to trypsin. Stroud *et al.* make use of the recent results obtained with α -lytic protease (an elastase-like serine protease), by Richards and his colleagues (*J. biol. Chem.*, **248**, 8306; 1973), using ¹³C magnetic resonance studies with selective labellings in suggesting that the proton acceptor is the aspartic acid, and not the histidine residue. The imidazole ring of this histidine, which remains neutral throughout catalysis, serves simply as a relay between the serine and the aspartic acid residues. It thus keeps

the carboxylate in a hydrophobic environment such that its pK is increased to 6.7. The advantage of this new scheme (which is at variance with hitherto widely accepted doctrine), is that it implies no charge separation during the formation of the tetrahedral intermediate; and is therefore on the face of it, to be preferred energetically.

Limited progress with quantum gravity

from a Correspondent

Most people would regard quantum theory and gravitation theory as two quite disjoint subjects; the first concerned with microscopic systems such as atoms, the second with objects of astronomical dimensions. Nevertheless, the marriage of these two unlikely bedfellows has fascinated a generation of physicists, for although quantum gravitational effects are deemed to be forever beyond the scope of direct experimental verification, the aesthetic appeal of a theoretical unification of these disciplines has always been compelling.

One of the driving forces behind research into quantum gravity is J. Wheeler (Princeton University), who has been visiting Britain during the past few weeks. His visit coincided with a conference on the subject held at the Rutherford Laboratory on February 15 and 16, and organised by D. Sciama (University of Oxford). The large number of participants testified to the extraordinary degree of interest in Britain for what is beginning to seem a theoretical obsession. With the sort of optimistic statements made recently by workers active in the subject, the conference might have been expected to produce convincing evidence of definite progress. In fact, it succeeded in generating a deep scepticism.

C. Isham (King's College, London) began with a review of the various levels of theoretical sophistication available to the would-be gravity quantiser, each level having its particular brand of ambiguities and problems. The presentation of technical results by M. Duff (University of Oxford) only served to confirm the extraordinary lengths which are necessary in order to make even minor progress. Pessimism mounted with evidence supplied by J. Taylor (King's College) that if gravitation is to be described by general relativity, then apparently it would be unrenormalisable in the usual sense familiar from quantum electrodynamics. That is, not only do infinite quantities appear everywhere in the theory, but they appear an infinite number of times for even the simplest type of process. Quantum gravity, said Taylor, "is dead".

If that is so, the subject was promptly resurrected by the following day with a series of talks by the 'fresh start' advocates. Adopting the philosophy that if the problems encountered on one level of the theory are insuperable, descend to another and start again, attempts were described in which the very structure of space and time are called to question. R. Penrose (Birkbeck College, London), supplemented by his student, G. Sparling, gave an account of his theory of twistors, but left a strong impression that the resulting formalism, whatever its intrinsic interest, is still very far from reality. A lot of comment was heard about starting from nothing and pulling all of physics out of a hat, but with no conceivable experimental framework to restrain their imaginations, the fresh starters continued to give the impression of drifting through a fairyland in which anything goes.

In marked contrast, work being carried out at less sophisticated levels does seem to be making some, if limited, progress. M. MacCullum (University of Cambridge) reviewed developments in quantum cosmology, which is an attempt to quantise the Universe considered (artificially) as a system with a small number of degrees of freedom. Undoubtedly the main attraction of the conference was a presentation by the indefatigable S. Hawking, now published in *Nature* (248, 30; 1974) on exploding black holes. This was based on recent work which shows that when quantised fields are examined in a curved space background metric, not itself quantised, remarkable effects such as the spontaneous creation of particles out of the vacuum may occur. According to Hawking, creation processes of this type would cause microscopic black holes to disappear in a burst of radiation. Exciting through this prospect may be, no plausible physical mechanism could be discerned which might lead to such a dramatic effect.

Last but not least, A. Salam (Imperial College, London) gave a most entertaining evening talk about almost everything, including evidence that the proton may have a finite lifetime, with all the consequences that may hold for the physics of the early big bang in cosmology.

Wheeler's own distinctive solution to the quantum gravity problem was described in his distinctive philosophical style to a packed gathering at King's College, London on February 27. Fortified with the belief that the ultimate equations of the Universe would reveal themselves by being "so beautiful and compelling" as to be obvious, Wheeler inclines to the view that space-time is built out of an underlying 'pre-geometry' in much the same way that elastic media are built out of atoms. Only the most tentative steps have been

made along this fascinating path, based on the notion that the underlying structure should allow description in the simplest possible terms—perhaps just the logic of a 'yes-no' decision. For John Wheeler, this is a jumping-off point into the realms of philosophy, for how is the decision to be made? The mind, which has always had an uneasy co-existence with the world of the quantum, occupies a central role in Wheeler's universe—a 'participatory' universe as he calls it.

Whether or not all this will eventually result in a type of unification of quantum theory with space-time or gravitation theory, that will satisfy the aesthetic tastes of the physicist, is anybody's guess. What does emerge from these discussions is first the rather practical point that the quantisation programme so far has very little to do with general relativity (the discussion is almost exclusively dominated by field theorists and particle physicists) and, second, that progress with a full theory of quantum gravity (or if necessary a restructured space-time) is unlikely to be achieved until much more understanding of quantum field theory at the less sophisticated level of the Hawking result has been achieved. But the last word should perhaps be Wheeler's: when asked whether a young student wanting to work on quantum gravity should be a relativist or a field theorist, he replied with careful deliberation that such a person would be better advised to find something other than quantum gravity to work on!

Regulating the amounts of RNA

from our
Molecular Genetics Correspondent

ATTEMPTS to define the systems which control transcription have for the most part concentrated on individual gene systems whose products can be identified. Yet equally important is the problem of how the cell adjusts the general amounts of synthesis of different classes of RNA in response to changes in its environment. That the relative extents of transcription of unstable (messenger) RNA and of the two classes of stable (ribosomal and transfer) RNA are set in response to growth conditions is well established in bacteria. The increased complexity of RNA synthesis in eukaryotic cells, together with the lack of mutants influencing the control of transcription, have made comparable studies more difficult with higher organisms. But in a study of mouse fibroblasts maintained in culture, Johnson, Abelson, Green and Penman now report (*Cell*, 1, 98; 1974) on the amounts

of different classes of RNA present in resting and growing 3T3 and 3T6 cells.

With both cell lines—each derived from the fibroblast but with different sensitivities to contact inhibition and therefore possessing different growth controls—the amounts of both rRNA and mRNA are greater in growing than in resting cells. With 3T3 cells, in conditions of growth, there is some 2.8 times more rRNA; with 3T6 cells, growing cultures display 1.6 times more rRNA than resting cultures. But the mRNA content increases to a greater extent with growth; in 3T3 cells the increase is 4.0 times and in 3T6 cells it is some 2.3 times compared with resting cells. Each class of cell therefore increases its mRNA content more than its rRNA content in response to conditions promoting growth, the extent of the increase being characteristic of the cell line.

When serum (containing fibroblast growth factors) is added to 3T6 cells maintained in the resting state, the cells enter a quasi-synchronous division, the first cells entering the S phase of DNA synthesis after about 11 h, so that the first divisions take place after about 24 h. During the transition from resting to growth state, amounts of both ribosomal and transfer RNA start to increase about 11 h after stimulation and then continue to accumulate in a strictly coordinate manner. But mRNA behaves differently, increasing much more rapidly; the ratio of mRNA to rRNA increases 50% in the first 6 h and ultimately doubles. The rate of protein synthesis follows the rate of mRNA synthesis.

These results raise several intriguing questions about the systems which regulate the general amounts of RNA in cultured fibroblasts. One obvious point is that, unlike bacteria which possess only one known RNA polymerase, in eukaryotic cells mRNA synthesis is catalysed by one enzyme in the nucleoplasm whereas rRNA synthesis is controlled by a different enzymatic activity in the nucleolar apparatus. At least two different enzymatic activities, located in different cellular compartments, must therefore be controlled if any changes are made at the level of transcription.

Although the content of mRNA doubles before DNA synthesis begins in the transition from resting to growth state, previous work has shown that the overall rate of synthesis of HnRNA (the nuclear precursor to cytoplasmic messenger RNA) does not increase by this time—this implicates some step in mRNA production subsequent to synthesis of the precursor as a control stage at which the level of mRNA may be controlled. And since the rate of protein synthesis increases in harmony with the increase in messenger content—that

is before the increase in ribosome content—ribosomes must be used more efficiently during this period; this is consistent with models in which the frequency of initiation of protein synthesis is established simply by the amount in the cytoplasm of messenger RNA.

Amounts of ribosomal and transfer RNA seem to be perfectly coordinated in fibroblasts, unlike the situation in bacteria in which the ribosome/tRNA content may vary over a ten-fold range. But since these two classes of RNA display different stabilities in the fibroblast, their coordinate amounts cannot be established at the level of synthesis; neither rRNA nor tRNA is stable in resting cells, where their half lives are different, and rRNA is stable in growing cells whereas tRNA is not—details of the stabilities of different classes of RNA, a critical parameter in constructing models for the regulation of their activity, are reported by Johnson *et al.* to be in preparation. But the apparent coordination of the relative contents of tRNA and rRNA must clearly require noncoordinate synthesis. And the general response of the cell to conditions which stimulate growth is to increase its capacity for protein synthesis, the level of mRNA relative to that of the ribosomes and tRNA itself increasing so that the cell makes a more efficient use of the protein synthetic apparatus. Establishing the mechanisms implicated in the control of and response to the different states of the cell represented by the division cycle is clearly fundamental to any understanding of cell development, emphasising the importance of studies such as these which cast a light on the general systems that regulate RNA production.

Role of terminal sugars in biological events

from a Correspondent

DURING the past few years glycoproteins have moved dramatically from the ignominious position they long occupied of taking up the last forlorn session on the last day of international scientific meetings, to the glory of fully fledged symposia. There is scarcely a biological phenomenon that has not been bombarded with every laboratory's collection of lectins. Another obligatory reagent for research at present is neuraminidase, the effects of which are legion: on the immunogenicity of tumour cells; on receptor sites for viruses, hormones and certain lectins such as wheat germ lectin which has weak affinity for N-acetylneuraminic acid residues or other lectins having sialic acid specificity, for example, from *Limulus polyphemus*; on cell-

cell interactions; on lymphocyte transformation, and so on.

The clear demonstration of a causal relationship between these various events and terminal sialic acid residues of glycoproteins or gangliosides is unfortunately less commonly obtained, though notable exceptions are binding of myxoviruses and certain lectins that clearly recognise this sugar and bind to it. Another candidate is the interaction of the natural peptide, tuftsin with polymorphonuclear leukocytes (Constantopoulos and Najjar, *J. biol. Chem.*, **248**, 3819–3822; 1973). The enhanced phagocytic capacity induced by tuftsin is abolished when cells are first treated with neuraminidase so raising the possibility that membrane sialic acid is the receptor site.

Perhaps the most convincing demonstration of a direct role of terminal sugar residues in the control of biological activity comes from Ashwell and his colleagues, who have shown that neuraminidase treatment of a large variety of soluble glycoproteins (including hormones) redirects these components from a normal circulatory pattern to one in which they are quickly cleared from the blood stream by sequestration in the liver. Ashwell and his collaborators have clearly identified receptor sites present on hepatocytes that recognise another terminal sugar, D-galactose, which is exposed as such after removal of sialic acid residues.

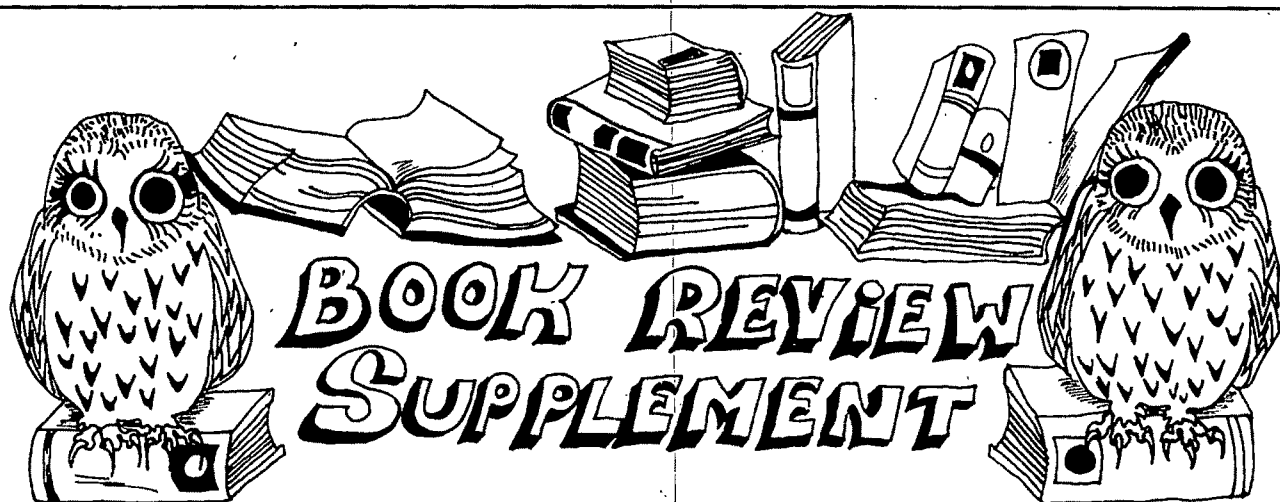
A possibly analogous situation is described by Sandford and her colleagues (*J. Immunol.*, **111**, 1071–1080; 1973) and may provide the explanation for earlier observations that the ability of a transplantable murine tumour to grow in certain foreign strains of mice is significantly reduced by prior treatment of the tumour cells with neuraminidase. Tumour cells treated with neuraminidase are rapidly killed by a naturally occurring antibody (probably 19S IgM) in the serum of resistant mice with specificity for newly exposed oligosaccharide terminals containing galactose residues.

The apparently widespread involvement of sialic acid terminals in biological activities, either directly or by virtue of a protective effect in 'hiding' other recognisable sugar terminals (galactose for instance), is a consequence of the ready availability of neuraminidases. Similar experiments using other glycosidases to point out possible functions of terminal sugars such as fucose have been hampered by the relative inaccessibility of active glycosidases of the required specificity. The recent report of Remold (*J. exp. Med.*, **138**, 1065–1076; 1973) suggests that it is only a question of time before other sugar residues are involved in biological events. Remold has studied the effect of the enzyme, L-fucosidase, on the interaction of macrophage inhibition factor (MIF) with its target cell.

MIF is a soluble glycoprotein of moderate size that is secreted by sensitised lymphocytes on exposure to antigen. It is so-called because of an ability to immobilise macrophages *in vitro*; macrophages are prevented in culture from migrating from the orifice of a capillary tube. The factor also performs other duties in enhancing the effector activity of macrophages in the immune response: for instance, in increasing their stickiness and phagocytic capability. It is relevant to point out that the carbohydrate unit of MIF seems to play a part in its activity since neuraminidase treatment inactivates MIF as shown 3 years ago by Remold and David. The unknown mechanism of action of MIF certainly involves as the first step, interaction of MIF with the macrophage surface. It is this step that Remold has now found to require intact fucose terminals on the glycoproteins of the surface membrane of the target cell.

First, guinea pig peritoneal macrophages, incubated *in vitro* with MIF in the presence of L-fucose at 100mM, fail to migrate as well as a control cell population and several other sugars have no effect. The inhibition by fucose is easily reversed by removing the sugar by dialysis. Second, when macrophages are treated with a specific glycosidase to remove L-fucose terminal residues from the surface, these cells do not respond to MIF but migrate normally. Clearly then the necessary fucose residues are part of cellular surface glycoproteins and are not part of MIF itself. Indeed MIF exposed to fucosidase in contrast to neuraminidase (or proteases) retains full biological activity. The inhibitory effect of fucosidase on macrophages is transient and the cells regain their ability to respond to MIF within a few hours, probably by replacement of the fucose-deficient surface membrane glycoproteins with complete molecules. The inference therefore is that the molecules containing receptor sites for MIF on the macrophage surface contain L-fucose residues and are manufactured by the macrophage population. Since L-fucose alone is inhibitory only at high concentrations, it seems likely that MIF recognises an extended oligosaccharide sequence terminating in an L-fucose residue in the receptor site on the macrophage surface.

The identification of this sequence and the question of whether it exists on a unique glycoprotein species of the macrophage surface, or alternatively if MIF will recognise a terminal L-fucose residue attached to any penultimate sequence remains to be determined. The competitive effect of L-fucose binding lectins on the activity of MIF would also be of interest and no doubt is under active study.



GENERAL BOOKS

Evolution and God

Darwin and his Critics. By David L. Hull. Pp. xii+473. (Harvard University: Cambridge; Oxford University: London, September 1973.) £9.25.

ONE might well ask whether a collection of the reviews of Darwin's *Origin of Species*, written shortly after 1859, could still be of any interest. Even a quick perusal of this volume answers this question in the affirmative; it shows how fascinating these reviews are and how amazingly pertinent to the present day. Even though written by scientists—contemporary reviews by clergymen are not included—they deal not only with questions of scientific evidence but raise a number of timeless problems such as the relation between science and a belief in the supernatural, questions of the correct method and philosophy of science, of the relations between biology and the physical sciences, and of the nature of scientific revolutions.

The Darwinian revolution occupies a unique position among scientific revolutions because, far more than any others, it caused a dramatic upheaval in the thinking of man. Only this can explain the bitterness, indeed sometimes downright nastiness, of some of the reviews. By his explicit analysis of all aspects of the diversity of the living world, Darwin forced every thinking reader into the uncomfortable position of having to make a choice in favour of one of the only three possible causal explanations of the living world: (1) continuing creation, that is the everlasting intervention of the creator in replacing species and faunas that had become extinct and in creating ever-new adjustments and adaptations; (2) a belief in the existence of evolutionary laws with a built-in teleology ordained at the

time of creation, that would lead to ever greater perfection and adaptation, and to an orderly replacement of faunas in the geological sequence, and (3) a strictly non-teleological evolution, governed by random variation and natural selection, without any recourse to supernatural intervention, even at the beginning.

To accept the third of these interpretations meant rejecting the other two. Thus, the Darwinian revolution was not merely the replacement of one scientific theory by another, as had been the scientific revolutions in the physical sciences, but rather the replacement of a world view, in which the supernatural was accepted as a normal and relevant explanatory principle, by a new world view in which there was no room for supernatural forces. For the devout this meant a world without God, and such a view was heresy if not blasphemy.

Even though the present volume limits itself to sixteen reviews by scientists, the defence of the view that the world is the result of creation and governed by finalistic laws is prominent in each of the twelve reviews critical of Darwin. The reviewers are particularly stung by the silent implication of Darwin's thesis that it is unscientific to believe in supernatural causation. Hence their strenuous efforts to prove that Darwin himself is unscientific. Forever they repeat the claim that Darwin provides no proof for his manifold assertions, ignoring the fact that they were employing double standards, since Darwin's probabilistic evidence was immeasurably stronger than their own assertions of continuing creation. Rather remarkably, about half of the opposing reviews were authored by physicists, mathematicians, or other non-biologists. Their arguments often reveal an extraordinary ignorance of biological facts which they attempt to

compensate for by accusing Darwin of having violated "the true method of science", that is a strictly inductive approach. Hull, a philosopher, treats this problem in detail in a seventy-five page introduction. He deals very perceptively with the role of philosophical concepts and methods in the Darwinian revolution, as they relate to essentialism, teleology, and the inductive method, and brings out impressively to what extent Darwin was ahead of his contemporaries even in these theoretical matters. Nothing could be more wrong than the immemorial assertion: 'Darwin was no philosopher'. Indeed, it was his philosophical rethinking which permitted Darwin to consider species as populations rather than as essences, a formulation that was the prerequisite for the adoption of natural selection as the directing factor in evolution. His empirical philosophy allowed Darwin to be satisfied with the probabilistic nature of his conclusions (against complete "certainty") and to adopt the hypothetico-deductive method.

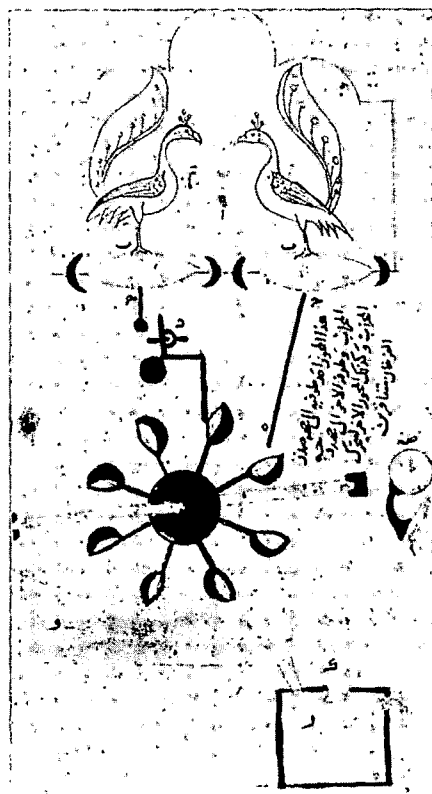
One must grant Darwin's opponents the validity of two of their objections. First, Darwin produced embarrassingly little concrete evidence to back up some of his most important claims. This includes the change of one species into another in succeeding geological strata, or the production of new structures and taxonomic types by natural selection. More importantly, although some of this has become apparent only in retrospect, Darwin either left totally unanswered certain serious problems like the origin of life and of new genetic variation, or he was himself confused about the problem as with respect to speciation, to the nature of variation, and to the role of isolation.

How far Darwin was nevertheless ahead of his contemporaries is clear not

only from the opposing but also from the supporting reviews (Hooker, Carpenter, Fawcett, and Wright). Since they also rejected supernatural intervention, they felt that Darwin's explanation was by far the best available one. But even these supporters were, on the whole, not too comfortable with natural selection. Nor were they thinking truly in terms of variable populations. To shift over to Darwin's radically new thinking was obviously difficult for anyone who had been raised in an era of creationism and essentialism.

The length of the reviews astonishes

Peacock water clock



IBN AL-RAZZAZ AL-JAZARI culminated the Muslim engineering tradition of the twelfth century. One of his mechanical devices was this clock which, with its decorations ran on water power. Water from an ornamental pool fell into a tipping bucket which filled and tipped every half hour. Hence by a system of pulleys the time on the clock face was changed, a peacock and a peahen were rotated, and the peacock whistled. The illustration shows another part of the machinery: a water wheel which in turning caused two young peacocks to 'quarrel'. The crank in the left-hand arm means that the chicks were tilted together or apart by the turning of the wheel. Al-Jazari's *Book of Knowledge of Ingenious Mechanical Devices* is translated and annotated by Donald R. Hill. (Reidel: Dordrecht and Boston, December 1973.) Dfl. 240; \$96.00.

a modern scientist. They average nineteen printed pages, even though the longest reviews (mostly of book length) by von Baer, Flourens, Kölliker, and Mivart are not included in this anthology (but short ones by von Baer and Mivart are). A book review in Darwin's day was an opportunity for the reviewer to write an essay on the subject matter of the book. Other reviews which were not included are listed in the bibliography.

The volume, in addition to reviews by eleven British authors, also includes five by foreign ones (Bronn, Pictet, von Baer, Wright, L. Agassiz). Curiously, Darwin was received much more favorably in America and Germany than in Britain. *Naturphilosophie* and a wave of extreme materialism had prepared for Darwin's enthusiastic reception in Germany. Unfortunately, however, Darwin was at once used as an ally in the fight against clericalism, particularly by Haeckel, and this rapidly led to a rather violent reaction, as reflected in the review by K. E. von Baer.

Hull has done a splendid job in editing these reviews. Each review is preceded by excerpts from Darwin's copious correspondence which deal with the reviewer, or more specifically, with the impact the review had had on Darwin. The editor then provides a concluding essay for each review in which the more important biographical details of the reviewer are presented, together with an analysis of his major arguments. The volume concludes with a detailed bibliography and a most helpful index. All in all an important and most stimulating contribution to the Darwin literature.

ERNST MAYR

The political scientist

Liberation and the Aims of Science: an Essay on the Obstacles to building a Beautiful World. By Brian Easlea. Pp. xiv+370. (Chatto and Windus, for Sussex University: London, November 1973.) £5.50 boards; £2.50 paper.

OVER the years, well meaning scientists and educators have fostered various 'integrating' or 'bridging' studies of natural science; and they have subsequently been disappointed when the intended purveyors of wisdom revert to type and become specialised research workers. As the title of this book proclaims, the author, a lecturer at the University of Sussex, has retained the courage of his convictions. Some may take this book as evidence for the blessings of pedantry. Others may see it (and the course it summarises) as providing a healthy dose of politics to science students; it might convert them to radicals, or at least to more self-aware (and hence effective) servants of the system.

In structure the book is a very thick sandwich: rather more than a third in the middle is on politics, mainly contemporary. The beginning is a very good exposition of the Popperist-Kuhn debates, with well developed examples from the Copernican revolution and non-relativistic solutions to the electrodynamics crisis of the early twentieth century. Both these examples point up the role of judgement in the acceptance of scientific theories, and the impossibility of any simple formula for validation. In this aspect the author's philosophy of science moves towards Polanyi.

Having established "commitment" as central to scientific work, the author uses it to introduce his analysis of social philosophy. He briefly studies Adam Smith, John Stuart Mill, Karl Marx and Alfred Marshall from this point of view, showing that "commitment", while explaining much about a thinker, is not necessarily in contradiction with the "objectivity" of his analysis. Keynes and his ideas are studied at greater length, and the section concludes with an exposure of "objectivity" in the social sciences.

The argument then moves to society itself, providing a Marxist critique of American capitalism and neo-colonialism. There is a new contemporary relevance in the argument by Heilbroner (quoted here) that although imports from the "underdeveloped" countries to ourselves might be small in proportion to the total, they act as "vitamins" to the economy. Since we are unlikely again to be so casual and patronising about our indebtedness to the poor nations, it is salutary to be reminded how recently such an attitude was shared by all but the radical fringe. The political discussion concludes with "Socialism in Crisis", showing all the warts but blaming them all on the unfortunate persistence of capitalism. Had the author provided an anarchist critique of socialism to match his socialist critique of capitalism, this part of the book would have been much enriched.

The book returns to science for its final quarter; here the critique is mixed socialist and eco-romantic. Easlea gives a fine survey of the "counter-culture", and a reminder of moral weaknesses in the history of the atomic bomb. Very near the end of the book is an analysis of J. D. Bernal's essay of 1929, *The World, the Flesh and the Devil*; showing the marriage of reductionist and Faustian themes that formed the basis of his commitment. As a man of influence in his century, and a late representative of a tradition still dominant, Bernal provides a most useful example. By contrast, Easlea believes in a beautiful world where science would be play, following William Morris more than Karl Marx.

Easlea's humanitarian commitment, translated into a certain orientation in politics, is overwhelmingly strong. Yet it does not destroy his objectivity; he is generous to all but the most reprehensible of his opponents and freely admits weaknesses and difficulties in the causes he holds as good. Any scientist browsing through it (starting with the excellent resumé at the end, a feature to be praised and emulated) will occasionally find himself shocked and annoyed, sometimes lost in an unfamiliar area of the author's broad competence, but never bored and frequently stimulated. The imposition of intellectual discipline on an intense personal vision has succeeded in this case; those who know how hazardous an operation this is, can congratulate the author on his achievement.

J. R. RAVETZ

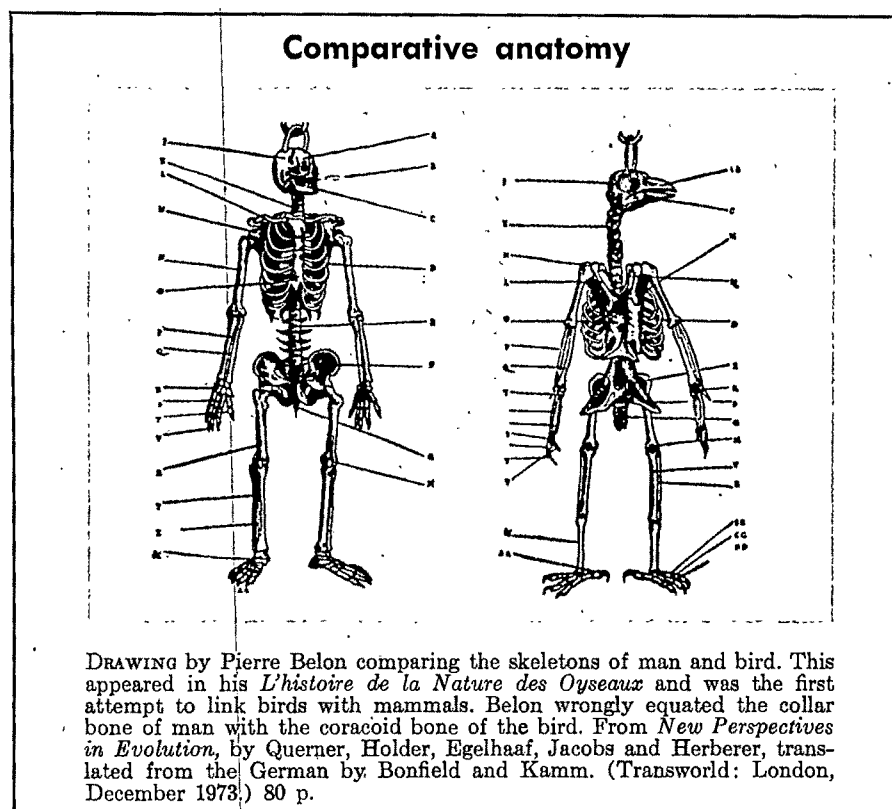
IQ and inequality

I.Q. In the Meritocracy. By R. J. Herrnstein. Pp. x+193. (Allen Lane: London, November 1973.) £2.50.

Inequality: A Reassessment of the Effect of Family and Schooling in America. By C. Jencks. Pp. xii+399. (Allen Lane: London, 1973.) £5.00.

JENCKS and Herrnstein present two interpretations of the problem of inequality. Whereas Jencks is primarily concerned with the sources of individual differences in access to the educational, social and economic benefits of American culture, Herrnstein is more concerned about the less immediate consequences of biological inequality for social structure in a meritocracy. Both authors base part of their work on what they regard, individually, as acceptable models for variability in intelligence. To some extent their models differ, consequently their overall assessments of the role of intelligence also differ. Herrnstein, partly because he has adopted a model in which there is considerable genetical variability for IQ, affirms the social relevance of IQ measurements. Jencks, partly by his acceptance of a model in which genetical differences are less important, suggests that the contribution of genetical variation in IQ to traits that have social consequences is proportionately less than is sometimes supposed, although more than many would admit. The authors seem to differ in their interpretation of data, the veracity of which they both seem to accept. Such disagreement as exists therefore, stems not from differences in their attitude to the data, but from differences in analytical procedure.

Herrnstein does not attempt to analyse data. He has accepted a model based on



Jinks and Fulker's biometrical genetical reanalysis of some of Burt's correlations for IQ. Although on this model some 80% of the variation in IQ may be attributed to genetical variation, a substantial part of this is due to the non-fixable contribution of dominance and to the increase in genetical variability arising from assortative mating. Jencks, on the other hand, provides a detailed reanalysis using the method of path coefficients and argues that the heritability of IQ is nearer 45%. In addition, he claims that different estimates of 'heritability' are obtained from different degrees of relationship and that there may be positive covariation between genotypic and environmental deviations: the so-called 'double advantage' phenomenon. Although Jencks suspects the presence of dominance, he does not treat the matter analytically, an omission which, as we shall see, could explain some of his unusual conclusions.

A fundamental weakness of Jencks's approach is his failure to make explicit the mathematical relationships between genetical paths in different pedigrees (path diagrams) and between different paths of the same pedigree. For example, not all the numerically feasible solutions given by Jencks in Table A-5 for the relationships between his paths g (between parental and offspring genotype) and h^2 (broad heritability) are genetically sensible. Genetical theory indicates that only solutions in which $g \leq \frac{1}{2}h^2$ for the parent-offspring covariation are genetically sensible. His failure to specify these restraints means

that equal weight is given to sense and nonsense answers. When we consider the amount of assortative mating and make allowance for the possibility of dominance the only estimates of h^2 consistent with a genetical model lie between 0.59 and 0.76 corresponding with Jencks's estimates of g of 0.25 and 0.30. Rather than supporting a lower heritability, therefore, on this basis alone Jencks's data are consistent with the value obtained by fitting a biometrical genetical model to all the data simultaneously (see Tables 1 and 2).

A further weakness of Jencks's approach is his failure to deal systematically with dominance. This can be seriously misleading for a trait such as IQ for which there seems to be considerable non-additive genetical variation. Dominance could account for much of the apparent heterogeneity between estimates of heritability obtained from different degrees of relationship. The ratios given by Jencks do not estimate the broad (or narrow) heritability in the presence of dominance and the inconsistencies to which he refers do not reflect haphazard inconsistencies in the data but the ordered departures from simple additivity which, within the limits of sampling variation, depend on the presence of a considerable amount of dominance (see Table 2).

Analytically, Jencks's procedure is inefficient. He estimates parameters from one set of data and uses these to correct estimates from other sets. This is not merely inelegant, it also yields the poorest approximations of the param-

TABLE 1 Observed and expected correlations for IQ

Relationship	r_{obs}	Burt r_{exp}	N	r_{obs}	Jencks* r_{exp}	N
Parent-child T	0.49	0.48	374	0.55	0.55	1250
Parent-child A	—	—	—	0.45	0.27	63
Grandparent-grandchild	0.33	0.28	132	—	—	—
Monozygotic twins T	0.92	0.92	95	0.97	0.97	50
Monozygotic twins A	0.87	0.83	53	0.75	0.68	19
Like-sex dizygotic twins T	0.55	0.56	71	0.70	0.59	50
Unlike-sex dizygotic twins T	0.52	0.56	56	—	—	—
Full sibs T	0.53	0.56	264	0.59	0.59	1951
Full sibs A	0.44	0.47	151	—	—	—
Uncle-niece etc	0.34	0.36	161	—	—	—
1st cousins	0.28	0.22	215	—	—	—
2nd cousins	0.16	0.11	127	—	—	—
Fosterparent-fosterchild T	0.19	0.10	88	0.28	0.29	1181
Unrelated T	0.27	0.10	136	0.38	0.29	259
Marital	0.3875	0.4133	(100)†	0.57	0.57	887

* correlated for unreliability and differences between sample means.

† Actual sample size not known.

T = living together; A = living apart.

eter estimates and could lead to the rejection of an otherwise acceptable model. Jencks may have been wise in eschewing statistics in a tentative analysis of heterogeneous data, but some attempt to use statistical criteria could have led to a more objective assessment of the confidence which others could place on his conclusions.

We have, therefore, subjected the correlations used by Jencks to a biometrical genetical analysis in which the expectations in terms of a model are fitted to all the statistics simultaneously so that the parameters are estimated from the full data set and the agreement between the observed and expected statistics after fitting the model can be tested. A parallel analysis of the full table of correlations given by Burt, only part of which has been hitherto analysed by these methods, has been carried out for comparison.

The data are given in Table 1. Normally we prefer to work with raw variances and covariances rather than correlations because the latter are far from normally distributed even in quite large samples and because valuable information is lost by standardisation to unit variance. A weighted least squares analysis of the two sets of correlations was conducted which has the advantage of efficiency and provides (given normality)

a test of goodness of fit of the model. Because of assortative mating the model is non-linear and a more complete discussion of the method and the model will be published elsewhere. By adopting a weighted least squares approach we have ensured that statistics based on small samples are given proportionately less weight in determining the final solution. As a result, the small samples of monozygotic twins reared apart, which have been criticised on several grounds, play a relatively small part in our analysis. In fitting the model simultaneously to a wide range of relationships we have evaluated its predictive validity in a broad context. Our model involves five parameters: an additive genetical component (D_R), a dominance component (H_R), a common environmental component (E_c), the marital correlation (μ) and the correlation between the additive genetical deviations of spouses (A). A sixth component, the specific environmental component (E_1), is obtained by difference at the end of the analysis.

This model differs from that quoted by Herrnstein by making E_c an environmental component shared by parents as well as offspring. This may lead to underestimation of D_R and consequent overestimation of H_R and A if unjustified. Making E_c applicable

purely to offspring results in a slightly poorer fit to Jencks's data. Our model differs from Jencks's in assuming that placement effects and the covariation of genotype and environment are negligible. The former assumption will lead to the overestimation of E_c in the event of placement correlations being significant, the latter assumption to failure of the model in the event of genotype-environment covariation being substantial. Such covariation is most easily detected by the analysis of raw variances. Jinks and Fulker were unable to detect it in their analysis of variances but the relevant sample sizes were small. Any analysis which does not make provision for an adequate test of the model may well discount some dominance variation as positive genotype-environment covariance and *vice versa*.

Burt's correlations are for final assessments and those given by Jencks include his corrections for unreliability and for differences between sample means. We have retained these corrections in order to yield results which are more directly comparable with those of Jencks. By treating such estimates as raw correlations we have, among other things, overestimated their precision and consequently regarded the analysis as more sensitive than is

TABLE 2 Results of model fitting

Parameter	$\hat{\theta}$	$\sigma_{\hat{\theta}}$	Burt χ^2	d.f.	P	$\hat{\theta}$	$\sigma_{\hat{\theta}}$	Jencks χ^2	d.f.	P
D_R	0.57	0.17	11.12	1	†	0.48	0.10	24.86	1	†
H_R	1.15	0.25	21.66	1	†	1.37	0.11	159.65	1	†
E_c	0.10	0.03	8.99	1	*	0.29	0.02	152.40	1	†
A	0.47	0.10	21.98	1	†	0.30	0.11	6.86	1	*
μ	0.41	0.08	17.28	1	†	0.57	0.02	630.87	1	†
E_1	0.08	—	—	—	—	0.03	—	—	—	—
Residual	—	—	8.96	9	0.5-0.3	—	—	6.63	4	0.2-0.1
Broad heritability	0.83	—	—	—	—	0.68	—	—	—	—

* significant at the 0.01 level

† significant at the 0.001 level

really the case. But whatever else may be said about the quality of the data, their quantity is such that our estimates are fairly precise and our test of the model fairly sensitive.

Table 2 gives the weighted least squares estimates of the parameters. The standard errors are approximate and assume that the deviations between observed and expected are small enough to be ignored. The expected values obtained by fitting the model to the data are given alongside the corresponding observed values in Table 1. Although individual deviations are sometimes large, the overall weighted sum of squared deviations, which is χ^2 if the observations are normal, is small in relation to the total weighted sum of squares of the observations.

Although the two data sets differ with respect to μ and E_e they are consistent for the estimates of D_n , H_n and A . There is some support for Jencks's conclusion that the heritability of IQ is apparently lower in the American studies than in Burt's British study. The best estimate of the broad heritability of IQ scores, however, for Jencks's data, is 68% which is 50% higher than the estimate Jencks has accepted. Since the model fits his data we cannot support his conclusion that the data give a heterogeneous picture of the genetics of IQ. Neither can we conclude that the data provide any evidence of genotype-environment covariation when proper allowance is made for dominance. On the contrary, when we allow for such covariation in the simplest way by adding to our five-parameter model one further parameter, r_{ge} , to specify the contribution of this covariation, no significant improvement in fit of the model to the data set is achieved. Furthermore, the weighted least squares estimate of r_{ge} is -0.31 with an approximate standard error of 0.20 . This is not only non-significantly different from zero but in so far as it is negative it has the opposite sign to the covariation postulated by Jencks in his model.

A small anomaly in the results of our analysis of Burt's data is that A is numerically (though not significantly) greater than μ . This anomaly is removed by stipulating that parents and offspring do not share developmentally important environmental features. The correlation between foster parent and adopted children then has to be accounted for partly by placement.

The analyses strikingly confirm Jinks and Fulker's conclusion regarding the importance of dominance variation. Even if we make allowance for possible overestimation this can best be explained only if dominance deviations at individual gene loci are large or if increasing dominant alleles are more frequent than their recessive counterparts. Coupled with the evidence for inbreeding depression, this suggests that IQ displays the pattern of genetical variation associated with a fitness character, that is, a trait which has been subject to a history of directional selection for increasing IQ score. Whatever else may be said about its social significance, IQ is clearly a trait of biological relevance.

Because much has already been made of the relatively low estimate of heritability argued for by Jencks, we have concentrated on the small part of his book devoted to this subject. We felt that it was important for the continuing discussion to establish that Jencks's American data do not in fact give a picture for the genetics of intelligence which differs in principle from that which has long been apparent from British studies. In so doing we have not done justice to the scholarly presentation and thought-provoking discussions contained in both Herrnstein's and Jencks's books, which have more in common than perhaps either author would admit to, and little that we would wish to dissent from. We are agreed that there are individual differences for IQ, that only a proportion of these differences might be removed by environmental manipulation, and that the

features of the environment that would require modification to achieve this have still to be identified. Those concerned with inequalities in our society might just as well resign themselves therefore to the fact that individual differences for IQ at more or less their present level will continue to be an important factor. In these circumstances, those who wish to see progress towards a more equitable society should turn their attention to the means of ensuring that the greater social and financial rewards of our society are not reserved for those who, whether through their genotype or environment, have a higher than average IQ.

J. L. JINKS

L. J. EAVES

Plan by computer

Computers and Socialism. By Stephen Bodington. Pp. 245. (Spokesman: Nottingham, 1973.) £4.00.

THE book considers, in broad terms, the impact that computers could have on society in the foreseeable future. Descriptions are in non-technical terms, and are aimed at the intelligent layman with an interest in computers and economics.

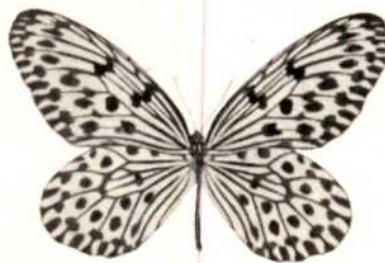
A chapter is devoted to an appraisal of modern computer technology. Although in the thirty pages involved one could hardly expect a complete survey, there are some omissions. Little is said of the difficulty in constructing large software systems, in spite of the fact that software now dominates hardware in cost terms. It is rapidly becoming apparent that our capacity to control such large systems is a limiting factor in the development of computers as a whole.

A major part of the book is devoted to the thesis that computers should permit better planning by provision of more accurate information. As is noted in the book, such changes are already taking place with the aid of linear pro-

Butterfly collection



a



b



c

THREE of the 7,000 butterflies illustrated in colour in *Butterflies of the World* by H. L. Lewis. (Harrap: London, February 1974.) £10.00. a, Female *Parides plutonius*, widely distributed in Asia; b, *Idea hypermnestra*, found in South America; c, *Marpesia chiron*, from Australia and southern Asia. Not to scale.

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gramming, PERT and even faster conventional accounting techniques. Several computer-based management information systems are now being sold commercially. The success of such systems largely depends upon the presentation and analysis of the basic information provided. Bodington looks forward to accurate economic modelling as the major planning tool. He hopes that a wide dissemination of information from such models will permit a decentralised power structure, although the political mechanism for achieving this is not discussed.

A surprising omission, in view of the lengthy discussion of modelling methods, is that of the world models of Forrester and Meadows. The significance of such models could be greatly increased if their reliability could be improved. But it does not seem likely that economic systems can be predicted with any accuracy (as the wide fluctuations in the basic commodity markets tends to show).

The book is well produced, although some of the formula and computer material is not correctly set. The subject matter is well presented, and since it is one of the first books in this area, it makes a useful addition to a library. I hope, however, that more penetrating analyses of the impact of computers will soon be available.

B. A. WICHMANN

Archaeologists entertain

Archaeological Theory and Practice. Edited by D. E. Strong. Pp. xiv+308. (Seminar: London and New York, August 1973.) £5.50.

THIS book is a *Festschrift* offered by his colleagues on the staff of the Institute of Archaeology in London to its director, Professor W. F. Grimes, after his seventeen years of service in that position. It is not, therefore, a survey of the theoretical structure of archaeology, as its title might imply, but an agreeable and wide ranging anthology, reflecting the considerable scope of interests of the staff of the institute, from Roman London (where much of Grimes's own work was carried out) to islands in southeast Asia, and from Acheulian hand axes to mediaeval technology. A particularly welcome feature is the inclusion of articles on photogrammetry and photographic techniques by staff in the Departments of Archaeological Photography and Archaeological Drawing and Survey.

Among the themes underlying this wide breadth of interest, a concern for environmental archaeology is perhaps the most prominent. Two further contributions are of very wide methodological interest. Professor J. D. Evans, Grimes's successor as director, writes

lucidly on his system of sherd analysis and sherd counts which he has used on the pottery from neolithic Knossos in Crete to very good effect, putting his findings on a firm quantitative basis. And M. H. Newcomer and F. R. Hodson describe a method here termed "constellation analysis", which permits the elegant comparison of different classifications of a given body of material, each classification being based on different criteria. A quantitative comparison is thus afforded of the results obtained from several independent approaches to the same set of data. In the example discussed the method has been applied to Newcomer's data on the burins from the upper palaeolithic levels at Ksar Akil in the Lebanon.

The human, almost anecdotal, interest of two contributions in particular catches the interest, for they are both at the same time contributions to scholarship. W. M. Bray, in "A page of *Punch*", reproduces a poem entitled "Monkey-ana". This is a lampoon, published in 1861, of the participants in the great British Association debate held in Oxford the previous year, when Huxley clashed so memorably with Bishop Wilberforce. In his discussion Bray brings out both the warmth in the scholarly world of the evolution controversy and, in an illuminating way, the extent to which the debate was reflected in the popular press as a whole.

The second of these delightful disquisitions is by the editor of the volume, Professor D. E. Strong, whose sad and sudden death in Turkey was reported shortly after the publication of this book. Professor Strong always accompanied his wealth of scholarship in Roman archaeology with a very modern wit, and in "Roman museums" he considers the Romans as connoisseurs and collectors of ancient art with a very contemporary awareness. The parallel with the pretensions and preoccupations of our own times is never explicitly drawn, but lingers engagingly below the surface. As scholarly as it is entertaining, this is a splendidly original and human contribution to a varied and wide ranging book.

COLIN RENFREW

Maths of Ch'in Chiu-shao

Chinese Mathematics in the Thirteenth Century: The Shu-shu chiu-chang of Ch'in Chiu-shao. By Ulrich Libbrecht. Pp. xxxi+555. (MIT: Cambridge, Mass., and London, 1973.) £11.25.

"ORIENTAL mathematics may be an interesting curiosity, but Greek mathematics is the real thing" (G. H. Hardy, 1940). Hardy's words were written before Needham's *Science and Civilisation in China*. The book under review is an

instalment of the volumes of detailed work needed before a successor to Needham, or at least a development of his mathematical section, can be attempted. But I don't think that either Needham or Libbrecht would have persuaded Hardy to change his views. The Greeks, lacking a decent notation for computation, developed mathematical ideas in a geometrical context. The ancient Chinese, having developed a notation of decimal place-values which allowed them to compute pretty efficiently and competently, developed their mathematics in a practical (though I know of nobody as practical as Archimedes) and pedestrian manner.

Perhaps changes here since Hardy's

In memoriam



GRAFTON ELLIOT SMITH (1871-1937) was a man of many parts; he was first and foremost an anatomist with a speciality for comparative neurology; later in life he became a determined and outspoken contributor to debates on man's physical and cultural evolution. These and the other interests of this extraordinary scientist, were reflected in the contributions to a symposium organised in November 1972 in commemoration of the centenary of Elliot Smith's birth by the Anatomical Society and the Zoological Society of London.

In the proceedings just published (*The Concepts of Human Evolution* (Symp. zool. Soc. Lond. No. 33) edit. by Lord Zuckerman (Academic: London, January 1974) £10.30; \$29.00), Lord Zuckerman—one of the small group of anatomists alive today who both knew and worked with Elliot Smith—introduces the man; there follows a medley of other relevant contributions and the volume ends with a thought-provoking and controversial session on "Elliot Smith, Egypt and Diffusionism".

death throw some light on this matter. The development of the computer is tending to strangle mathematical thinking among all but the brightest of its users: so one should not be surprised that the computational efficiency of Chinese mathematics tended to inhibit mathematical inventiveness except in the case of brilliant individuals not part of the State's official mathematical bureaucracy. Ch'in Chiu-shao (probably 1202-1261) was one of four such now known to have lived, and worked independently, within half a century. The *Shu-shu chiu-chang* is his mathematical legacy—a treatise with problems and answers intended for practical use. Ch'in, a soldier of fortune and administrator, was a colourful and dubious character who will doubtless, when Chinese mathematics is better known in the West, be included in the gallery of such characters with, for instance, such old friends as Cardano and Galois. The treatise is in nine chapters: apparently a significant structure already used in the *Ch'iu-chang suan-shu*, the standard textbook which in Ch'in's time had already been in use for about a thousand years (less than the time during which Euclid dominated the West!). The contents of Ch'in's book are in many ways similar to the earlier one, but the problems are harder and the fruit of a considerable individual development. But there are some things not to be found in the earlier work. For instance an algorithm for solving the so-called Chinese Remainder Problem (for which Ch'in is at present best known) is the most important. There is also what Dr Libbrecht, doubtless correctly, takes as clear evidence that Ch'in had completely anticipated Horner's method for the solution of equations. Ch'in was apparently so pleased with this method that he deliberately and unnecessarily complicated the solution of one problem in order to demonstrate his virtuosity with an equation of the (unnecessarily high) tenth degree.

Ch'in's book was intended, as the conventions of Chinese mathematics at the time required, as a practical treatise and not as a mathematical text as we know it: it was rather a cookery book, but the recipes were subtle and based on much deeper mathematical thinking than was expressly used in the text. It is for this reason that Dr Libbrecht's research into the book and its background is so valuable. At first sight Libbrecht's book is not an attractive work: it is written as a serious and scholarly study, not for easy reading, but to serve as a start for the accumulation of the knowledge necessary to write a more easily read work. But the book grows on one with deeper acquaintance; its integrity and thoroughness are totally convincing. Clearly Dr Libbrecht's second career as an ori-

ental scientific historian (his first was as a teacher of mathematics) is destined to be brilliant and rewarding. The chapter on the socioeconomic information to be derived from Ch'in's book raises high hopes of interesting future developments.

D. B. SCOTT

Sussex replies to Rome

Thinking about the Future: a Critique of the Limits to Growth. Edited by H. S. D. Cole, C. Freeman, M. Jahoda and K. J. R. Pavitt. Pp. 218. (Chatto and Windus (Sussex University): London, 1973.) £3.00.

Has the development of the energy crisis in the past few months strengthened the argument of Meadows's *Limits to Growth*, and weakened the trenchant critique of it, *Thinking about the Future*, by the University of Sussex Science Policy Research Unit team?

The fact that oil reserves are finite has no direct bearing on the Arab use of the oil weapon. It has depended simply on Arab states controlling a proportion of existing production capacity large enough to control the market. The long lags in exploration and drilling and the provision of transport elsewhere has made their action effective in the short term. It has at a stroke, and by design, raised oil prices to the level where as Surrey and Bromley point out in chapter 8, alternative sources of energy become economic. They further remarked, writing early in 1973, "In addition to the political uncertainties surrounding the future of Middle Eastern oil, some of the small Arab states which are large oil producers may decide to conserve their oil reserves until prices rise, rather than accumulating large foreign exchange reserves which are subject to depreciation by inflation and major currency re-alignments".

Peccei, the founder of the Club of Rome which sponsored *Limits to Growth*, has said that the political factors omitted from the study exacerbate the problem, and these political factors have brought forward the threat of doomsday. But it is equally possible to argue that political factors, as one aspect of the flexibility of human response, have brought forward action on energy supplies in time to deal with supply limitations before they became physically acute. Politics, for all its frustrations, can make society more adaptive than its dynamic systems model, not less.

Likewise within the United Kingdom, whatever the political and economic consequences of the miners' industrial action in the short term, the long term effect can be to secure a more even distribution of wealth and power within one industrial society, thus averting the

build-up of greater tensions in the future.

This is not to argue that all is for the best in the best of all possible worlds. But it does raise the question whether on population, food, resources, and pollution *The Limits to Growth* addressed the right problems at all.

The Sussex team, taking *The Limits to Growth* at its own valuation, reviews the evidence used and the structure of the model, and concludes they do not justify the conclusions drawn. As a bonus they put *Limits* in its ideological perspective, against the background of the history of economic thought, the movements of environmentalism and technocracy, and the processes of social change. On evidence and ideology the critique is strong. Experts in particular fields will disagree about the realism of some of the assumptions tried by the Sussex team but they will also criticise those made by Meadows. On the methodology of modelling systems of uncertain, tenuous and changing structure however, the Sussex critique is less sure footed. *The Limits of Growth* is deficient in having ignored the hard won experience of econometrics, stochastic estimation and control theory, and more general adaptive systems. Forrester and Meadows quite fairly argue that this systems apparatus as it stands cannot draw conclusions from the sparse information available on world environmental problems. But combined with a perception of the real world—Meadows's or anyone else's—it does give a more realistic approach to practical policy making than does the crudities of "systems dynamics", which is no more than the solution of simultaneous dynamic equations. The reason for this is that a wider systems theory illuminates the behaviour of indeterminate systems partially controlled with partial knowledge of system behaviour.

The Sussex critique does not touch this problem of adaptive behaviour beyond pointing to other factors in social change. There are these other factors, but their effect manifests itself in the indeterminacy, not the incoherence, of physical systems when viewed as systems on their own. But the physical systems are important enough to warrant the fullest analysis. For example the Sussex team is well equipped to analyse the evidence on the time lags and uncertainty in industrial innovation, a crucial factor in the ability of industrial society to adapt and survive. Perhaps without the inhibitions of criticising other work, but stimulated by the courage, even the sheer impudence, of *The Limits of Growth*, the Sussex team will contribute further to the vast task of building a genuine discipline of social systems analysis.

J. W. BRAY

PHYSICAL SCIENCES

Splitting atoms

Nuclear Fission. By Robert Vandebosch and John R. Huizenga. Pp. xii+422. (Academic: New York and London, November 1973.) \$29.50.

Fission is one of the most peculiar and important phenomena in the whole of nuclear physics. If the constants of nature had been slightly different it could not have occurred at all, and the history of the world after 1940 would have been completely changed. Yet in spite of its great practical importance it occupies a very peripheral position in nuclear physics itself. Concepts from the rest of nuclear physics are used to understand fission, but until recently studies of fission told us very little about the nucleus. By far the most important effect of fission on nuclear physics was indirectly through its applications. These led to the growth of vast industries and this so stimulated nuclear physics and made such large resources available that the subject is now several decades further advanced than it would have been if fission had never taken place.

One of the results of this situation was that although most people almost identified fission with nuclear physics, most nuclear physicists neither knew nor needed to know much about the fission process.

This situation has changed dramatically in the past few years with the discovery of a whole range of phenomena including shape isomers, intermediate structure and the double-humped potential barrier. These exciting developments have greatly stimulated research on fission and a much more detailed understanding of the process is now being obtained. From an entirely different direction the technical advances in heavy ion physics have made it possible to study the collision of heavy nuclei with each other, and thus to investigate the complementary fusion process.

The new knowledge has now consolidated to the extent that an extended survey is timely, and this book by Professors Vandebosch and Huizenga fills the need admirably. Both authors have studied fission for many years and are in a position to present a detailed and authoritative account of present knowledge.

After a brief historical survey, they summarise the liquid drop and single particle models, and their application to nuclei of increasing deformation. The Strutinsky model combines the two by superposing the variations in nuclear properties from nucleus to nucleus due to their shell structure on the average

behaviour given by the liquid drop model to give a detailed and accurate overall theory. This model is described in detail and applied to the systematisation of fission barrier heights and other features of fission.

Subsequent chapters consider the present state of knowledge on spontaneous fission, fission widths obtained from neutron resonance studies, the properties of low-lying levels of transition nuclei, the angular distributions of the fission fragments as a function of excitation energy and the competition between fission and neutron emission.

Fission can be induced by photons, deuterons, tritons and many other particles, and a study of the corresponding cross sections can give information about the fission potential barrier. Much is being learnt about the way a nucleus deforms on the way to fission, and the mass and density distributions as the separation takes place. The kinetic energy released in fission is of both practical and theoretical interest, as well as the distribution of mass and charge among the fission fragments. All these aspects are carefully described, and the three final chapters are devoted to the prompt neutrons from fission, ternary fission and the gamma rays emitted from the primary fission products.

Full references to relevant researches are given at the end of each chapter, enabling particular topics to be followed up in detail. This is a well balanced and comprehensive survey of our present knowledge of fission that fills a real need. It will be of great value not only to those studying fission itself but also to other nuclear physicists who find that due to its growing academic importance they now have to repair a conspicuous gap in their knowledge.

P. E. HODGSON

All people of the earth

Implications of Continental Drift to the Earth Sciences. Edited by D. H. Tarling and S. K. Runcorn. Vol. 1, pp. xvi+1-622; vol. 2 pp. xiv+623-1184. (Academic: London and New York, April 1973 and November 1973.) Vol. 1 £13.80; vol. 2 £13.00.

ON the face of it, Earth scientists can hardly be accused of failing to respond to social need, defined in this context as material demand. Geologists, geophysicists and geochemists have long been associated with prospecting and the discovery of minerals and fossil fuels; their brethren with eyes on higher things are now achieving ever greater success in the understanding and prediction of

atmospheric phenomena, much to the benefit of agricultural and other pursuits; and a careful search might even show that something has been contributed to man's physical welfare by palaeontologists. But at the other end of the spectrum there is another, rather more glamorous and generally quite distinct Earth science community whose task it has been to elucidate the nature of the Earth for its own sake. In the purely intellectual sense the achievement of the latter group has been immense; to its various members we owe what has come to be known as the revolution in the Earth sciences and all that it implies in vindication of Thomas Kuhn's paradigmatic hypotheses. On a less esoteric plane, however, the material benefits which a society increasingly suspicious of science might conceivably expect from the scale of its investment have been somewhat less apparent.

For this last reason, the Newcastle upon Tyne (April 1972) conference on the "Implications of Continental Drift to the Earth Sciences" was, despite titular ambiguity and curious grammar, welcomed by some as a sign that the purer Earth scientists were beginning to perceive their social responsibilities. On the whole, however, this proved to be a naive expectation even though in other respects the conference was rightly pronounced a success. Something like 20% of the papers presented were devoted to background reviews of palaeomagnetism and sea floor spreading; about 35% dealt with the palaeontological, palaeogeographic and palaeoclimatic implications of these phenomena; and a similar proportion was concerned with geological implications in terms of continental and oceanic evolution, rifts and rift margins, and continental edge structures. What these figures and headings demonstrate on the credit side is that Earth scientists of all disciplines are now talking to each other to a quite remarkable degree, almost all apparently having accepted the general validity of the new global tectonics and the need to interpret both new and old data in those terms. But it is equally clear, at least as far as this conference was concerned, that few people are yet able or willing to see their work in terms of its natural resource implications. Of the 87 papers involved only six—a disappointingly low proportion—were specific enough to be classified under the general heading of "economic significance", although it could be argued with some justice that many of the more geologically inclined presentations form the essential base for subsequent, more applied studies.

The two books under review are a record of the proceedings of the New-

castle conference, and as such reflect both the merits and faults of the conference itself. At the same time, however, they present problems of their own. I was about to say that they are the academic equivalent of the book of the film, but on reflection it would be more true to say that what we actually have here in part is the book of the film of the book. In other words, no small proportion of the material presented has been published before in other forms.

It is time that the pressures—of tradition, commercialism, the ego, or whatever—to manufacture tomes such as these were resisted more often, not least because the result is self-defeating. It is quite reasonable, of course, that conference organisers should seek to make available to nonparticipants something of what the privileged few have found useful or enjoyable. But when such proceedings are produced incompleteness may be a virtue. It would have been far more profitable for everyone if Tarling and Runcorn had commissioned a small group of the best scientists and writers involved to distill the essence of the 87 original papers into a book of 200 pages or so, avoiding overlap, the bad, the indifferent and the peripheral, and perhaps even restricting the coverage. People, rather than merely libraries, would then have bought the book and read it. As it is, "so it must be, for now all length is torture". As Mark Antony might say.

PETER J. SMITH

Experimental X rays

X-Rays in Atomic and Nuclear Physics. By N. A. Dyson. Pp. xiii+380. (Longman: London, December 1973.) £8.

THIS book is the logical successor to Compton and Allison's *X-Rays in Theory and Practice*. The author emphasises the constant interest that has been maintained in the subject since the discovery of X rays by Roentgen in 1895, and points out that the succeeding years can be roughly divided into three eras, each twenty-five years long. The first of these, from 1895 to 1922, saw the laying of the foundations, and a multitude of experiments were performed with the prime purpose of elucidating the properties and behaviour of X rays. The second period, 1922 to 1945, was involved with the development of X-ray spectroscopy, and the design and construction of sensitive detection systems. The third period, from 1945 to the present day, has been concerned more with nuclear properties and with the physics of the stars. It is not often that a single branch of physics has kept up its momentum over such a long period and has, at the same time, been able to generate such important and wide-ranging offshoots as X-ray

crystallography, Auger spectroscopy, and Mössbauer spectroscopy.

After a brief historical introduction, the author covers his chosen field in six chapters: the continuous X-ray spectrum; characteristic X rays; experimental techniques for the study of X rays; the absorption and scattering of X rays; X rays in radioactive decay; and some additional fields of X-ray study. There follows a number of appendices giving numerical data, a comprehensive bibliography arranged chapter by chapter, and finally a subject index.

The bias of the book is towards the experimental side which is understandable, since much of the physics of X rays is well understood and documented. The chapter concerned with experimental techniques is particularly notable for its wealth of detail for the construction of a variety of devices for the detection of both hard and soft X rays. Each chapter is copiously illustrated with diagrams emphasising the nature of the results that can be obtained in the particular area under discussion.

The last chapter is in some ways the most interesting one because here the author changes pace and selects various areas in which X rays have been used (such as various sorts of microanalysis), are being used (such as the isotope effect in absorption and emission), or are likely to play a dominant part (such as plasma physics and astrophysics). When, to all this, is added such important areas as medical physics and industrial radiography, it is clear why the author's mood seems to be so buoyant and optimistic. Certainly his claim that not only have X rays had a great part to play in the past, but are likely to continue to do so is certainly justified.

The book is economically written, well illustrated and carefully produced. It should be made available in all scientific libraries, and should find a place on the shelves of any individual who is interested in the production, properties, and applications of short wave-length radiation.

P. J. WHEATLEY

All dating is relative

Recent Earth History. By C. Vita-Finzi. Pp. xii+138. (Macmillan: London and Basingstoke, October 1973.) £3.00 boards; £1.50 paper.

THIS slim volume has an inappropriate title, for this relates rather to the author's original intention "to write an account of the physical changes undergone by the earth during Holocene times . . .", as he states in his disarming preface. This book presents the contention that chronology should be based upon dating and not upon stratigraphy,

that is, that dating throughout earth history should be chronometric and not relative.

The author quotes the anonymous reviewer of an early draft of this book that "Palaeontology . . . and stratigraphy are humble and intellectually unexact-ing and nothing written by [the author] or anyone else can change this for a long time". Although obviously someone who knew very little of modern palaeontology and stratigraphy, that reviewer was probably correct in concluding that such a view would not be modified by this book's contents.

The author apparently understands that quantitative dating depends upon the resolving power of the particular method, but a failure to deal satisfactorily with Oakley's point that "All dating is in a sense relative", renders the book less than effective for its purpose. The dustcover's claim that the book is "clearly written and logically argued" is perhaps rather too obviously a commercial.

EDWARD A. FRANCIS

Many bodies

The Many-Body Problem. By W. E. Parry. (Oxford Studies in Physics.) Pp. x+217. (Clarendon: Oxford; Oxford University: London, December 1973.) £6.25.

DR Parry, in writing this book, had a very clearly defined and limited aim to which he has stuck rigorously. Assuming that his readers would have a knowledge of basic statistical physics and of Dirac's approach to quantum theory, he provides them with a large number of the mathematical techniques currently used for dealing with many-body problems and shows how they are related. Applications are introduced to illuminate the mathematics but only for this purpose. Readers are referred elsewhere for a fuller study of specific physical problems. The result is a well balanced, comparatively slim book containing a wealth of methods which include second quantisation, perturbation theory, Green functions, correlation functions, linear response theory, partial summation of diagrams, equations of motion, the drone-fermion representation in magnetism and the use of the variational principle.

Despite the large number of techniques introduced, the book has a uniformity which makes it easy to read and to relate one part to another. The point of departure (one I favour) is temperature-dependent perturbation theory for the grand partition function and other techniques are related to this. Expansions for the properties of the ground state are derived from this as a special case. This approach makes the

book particularly useful for solid state and liquid physicists but, perhaps less so for nuclear physicists.

The whole is characterised by great care and thoroughness. Motivation and the main line of attack are always made clear. Very rarely does a "let us consider" leap out at one from the dark. Despite this, Dr Parry does not shirk the difficulties. He provides full proofs of the various basic theorems including the sometimes tricky points but the mathematical completeness does not obscure the main argument. This makes the book, within its limit, self-contained. The exercises placed throughout the text enable the reader to test his progress. The approximations introduced, especially in the partial summation of diagrams, are carefully discussed. The result is a successful realisation of the author's aims and a book through the pages of which one's research students can safely be encouraged to roam.

G. RICKAYZEN

Prolific mathematician

The Art of Counting: selected writings. By Paul Erdős. Edited by Joel Spencer. (Mathematics of Our Time, vol. 5.) Pp. xxiii+742. (MIT: Cambridge and London, 1973.) \$22.50.

THIS handsome volume contains a selection of about 80 papers from the prodigious output of Paul Erdős. It was produced to celebrate the 60th birthday of this peripatetic genius, and the selection has been made with a view to illustrating his unique contributions in combinatorics. In this latter respect the editor's choice is only partially successful (and the title is somewhat deceptive); for as Turan points out in a short introduction, combinatorial ideas have characterised Erdős's work in other fields too, and notably so in the theory of numbers. There is a glimpse of this in the papers concerned with latin rectangles, especially in the joint paper (314) with Chowla and Straus where a famous conjecture of Euler about pairwise orthogonal latin squares is shown to be just about as false as can be by means of quite deep arithmetical arguments. But this is not intended as a criticism but rather as an encouragement to other devoted friends of Erdős to set about collecting other portions of his work, not only as a tribute but also as a great service to scholarship.

A charming essay of reminiscences by Szekeres introduces chapter 1, which contains a seminal paper by Erdős and himself demonstrating for the first time the power of Ramsey's theorem; and this is also the theme of chapter 8. Chapter 2 contains four survey articles in which Erdős poses and discusses unsolved problems in his own inimitable style, and in the process charts the de-

velopment of the subject. His matchless ability to generate problems and conjecture results, and his unselfish willingness to share his mathematical concerns with anyone prepared to have a go, have inspired his contemporaries, great and small alike, for many years; in these lectures we come as close as we can in print to his essential mathematical personality. There are chapters on the colouring of graphs, on extremal graph theory, on systems of sets, on block designs, on tournaments, on information theory and on random objects; in each there is food for thought for years to come.

There is no sample from the important work of Erdős with Rado and Hajnal on the partition calculus, but this work is to be the subject of an independent book. There is also reference to another forthcoming book, by Erdős and Spencer (the editor) on probabilistic methods in combinatorics; these play an important part in many of the papers and lead to some remarkable existence theorems. Erdős's ability to grasp the essentials of a subject, or a theorem, and to apply them in other contexts is well illustrated by the striking use he has made of probabilistic arguments in many fields.

Each chapter is prefaced by a short editorial commentary but there are few textual corrections and there could have been more information about the subsequent history of some of the problems that Erdős has proposed over the years; there is the occasional reference, but sometimes only to the author's name. At the end of the book is an invaluable bibliography of all of Erdős's papers up to the present—some 600 papers in all; it is quite clear what his target should be for his 100th birthday!

H. HALBERSTAM

Gas chromatography

Techniques of Combined Gas Chromatography Mass Spectrometry: Applications in Organic Analysis. By William McFadden. Pp. 463. (Wiley-Interscience: New York and London, 1973.) n.p.

Gas Chromatography in Inorganics and Organometallics. By G. Guiochon and C. Pommier. Translated by STS, Inc., Ann Arbor, Michigan. Pp. viii+332. (Ann Arbor, Michigan; John Wiley; Chichester, December 73.) £10.

New Developments in Gas Chromatography. Edited by Howard Purnell. Pp. vii+408. (Advances in Analytical Chemistry and Instrumentation: vol 11.) (Wiley: New York and London, 1973.) n.p.

THE combination of gas chromatography and mass spectrometry is the most powerful tool yet devised in the

analytical chemistry of volatilisable organic materials, and there has been a need for a practically oriented book which would serve as a guide to the two techniques and their interrelation. McFadden's book seems to meet this need admirably. Both mass spectrometry and gas chromatography are introduced satisfactorily, but the technical emphasis is clearly stronger in the former so that the book will be most useful to those with some background and experience in gas chromatography. The interfacing of the two techniques, one vacuum and one atmospheric but otherwise beautifully compatible, is discussed in depth together with the methodology of operating them together and the role of computers. The main part of the book is based on a regular course of lectures and this seems to have given it an extra coherence and clarity. In a happy choice of method, the last chapter is a series of some sixteen short contributions by various authors each covering a different field of application or specialised technique.

The second book is essentially a translation of the excellent French text of Guiochon and Pommier on "Gas Chromatography in Inorganic Chemistry". Though the applications of gas chromatography in organic chemistry have been legion, those in inorganic chemistry are much more restricted. The whole field and its problems and successes are most clearly reviewed and considered in this work. After a short introduction to gas chromatography itself there follow discussions of analytical separations achieved in the fields of inorganic gases, halogens and non-metallic halides, metals and metal halides, hydrides, organometallic compounds, metal chelates and isotopes. Finally, there is a brief account of non-analytical applications such as the measurement of thermodynamic parameters and preparative chromatography. It is a pity that the translation is rather uninspired and the proof reading somewhat slipshod.

Purnell's *New Developments* is an interesting collection of seven specialist articles, most of which lie outside the realm of analytical chemistry suggested both by the title of the series with which this book is associated and indeed by the most common applications of the technique. The authority and personal emphasis which characterise most of the articles reflect the fact that they are written by recognised experts on their own fields. The first is a review of the many forensic applications of gas chromatography. The second provides an admirably clear introduction to the jargon and use of digital computers. Four articles then concentrate on the study of various processes of physicochemical interest by

gas-chromatographic methods; namely phase changes which occur in materials used as stationary phases, polymer structures and interactions (by inverse gas chromatography), the determination of the formation constants of complexes, and the kinetics of chemical reactions (by using the chromatographic column as a reactor). Finally, there is a timely, well balanced and dispassionate account of gas chromatography on the production (as distinct from the preparative) scale. This particular package may seem at first sight to have a rather narrow appeal, but I found it both satisfying and curiously homogeneous: it certainly provides a stimulating and very up to date introduction to the wider aspects of gas chromatography.

C. S. G. PHILLIPS

Physics with computers

Computational Physics. By David Potter. Pp. xi+384. (Wiley-Interscience: London and New York, October 1973.) £5.50 cloth; £2.50 paper.

THE widespread use of computers in problems of physical science is enabling investigations to be carried out which are not subject to some of the restrictions of mathematical analysis. As a consequence some, who have not given adequate attention to the difficulties of interpreting the voluminous output of computers, have consigned analysis to the back room. It is therefore pleasant to welcome a book which shows that good analysis and sound physical thinking must go hand in hand with numerical technique if computational results are to have any validity and reliability. The introduction, at least, should be compulsory reading for any physical scientist before he embarks on an extensive and expensive computer program. He will also find much that is well worth reading in the rest of the book but he must not expect to find a discussion of every problem of interest to him.

Although the book is called *Computational Physics* it really deals only with difference methods. There is no mention of finite elements or integral equations at all. Numerical analysis, as such, is confined to three chapters so that some topics are discussed more cursorily than desirable. Some places where fuller treatment is required are: interpolation and its difficulties; the fast Fourier transform; Poisson's equation when the boundary condition is not of Dirichlet type; eigenvalue methods. In general, the reader seeking the whys and wherefores of numerical techniques for ordinary and partial differential equations will have to consult other texts such as those of Lambert and Mitchell.

The book is not really suitable for

undergraduate teaching, partly because there are no exercises and partly because so many ideas creep in unannounced; for example, spectral radius, tensor inner product notation, Poisson brackets, sodium chloride mesh (note also that the definition of spectral norm is unsatisfactory).

Nevertheless, postgraduate workers will find this an extremely useful book to have for several reasons. It collects together material on numerical attacks which is not readily accessible elsewhere and provides an extensive bibliography covering many fields of application. Throughout there is careful discussion of the physical and mathematical approximations undertaken in reaching a stage where the computer has a reasonable chance of supplying a satisfactory answer. The relative merits of alternative approaches are also described.

Chapters 5 and 6 are devoted to the motion of numbers of particles, with and without field averaging, as in the collisionless case in plasmas and astrophysics while compressible hydrodynamics is an example of collisions. Long range quantum forces appear in chapter 7. The distribution function approach and Vlasov's equation occupy chapter 8. Chapter 9, dealing with hydrodynamics is the longest. It shows how the particle-in-cell method can display the development of Helmholtz instability and the creation of a von Karman vortex street; the marker technique for moving surfaces and breaking waves is also given (though the absence of Zabusky's work on the non-linear interaction of waves is a notable omission).

The complex nature of compressible flow is clearly brought out, and, in chapter 10, the related problems of magnetohydrodynamics are emphasised. Evidently, some new good ideas are going to be necessary before the computer can cope adequately with the diverse phenomena of shock waves and other large amplitude disturbances.

Well printed, the book is good value and will be appreciated by researchers.

D. S. JONES

Life in glacial Mexico

Das Mexiko-Projekt der deutschen Forschungsgemeinschaft. 6: Geologische und paläontologische Untersuchungen im Valsequillo bei Puebla, Mexiko. By E. W. Guenther, with H. Bunde and G. Nobis. Pp. 177; 14 plates. (F. Steiner Verlag: Wiesbaden, 1973.) DM. 69.

THE Mexico project of the German Research Association is a regional study of the Puebla-Tlaxcala Basin, with man in his local environment, from the Stone Age to the present, as its central theme. The present work concerns the geology and palaeontology of a small area some 10 km south of the City of Puebla.

Bird's eye view



PHOTO-mosaic of the west coast of Schleswig-Holstein. Taken from *The Earth from Space* by J. Bodechtal and H.-G. Gierloff-Emden, translated by H. Mayhew and L. Evans (David and Charles: Newton Abbott, January 1974; £6.50), this is in fact one of the few illustrations in the book not taken from space. The importance of this kind of mosaic is that a series of photographs on a scale of about 1:20,000 can be combined and reduced in size to give a picture with scale corresponding to satellite photographs; it is just possible to bridge the gap between satellite photographs and ordinary aerial photography.

Valsequillo owes its importance, in this context, to finds of undoubted stone implements in association with remains of extinct mammals, at horizons which though still not securely dated, suggest dates of the order of 40-50 thousand years BP. If this is the case they might be of an age some 10 thousand years earlier than any other evidence for the presence of early man in the New World. The present work contributes new facts and considered judgments of their implications to the basic disciplines of geology and palaeontology, on which any final decision as to the date of the human occupation must ultimately rest.

The palaeontological finds, apart from those of equids and elephants, have been dealt with elsewhere by other authors, but their results are summarised by Guenther in the introduction. Carnivores represented are coyote, dire wolf, a sabre-toothed cat and a very large bear, *Arctodus simus*. One fragment of a tapir is the only perissodactyl other than the horses. Remains of a peccary, *Platygonus cf. compressus*, were found in several examples. Camelids were not uncommon and two species of *Camelops* and one *Tanu-*



The Solar Chromosphere

R. J. BRAY AND
R. E. LOUGHHEAD

International Astrophysics Series

February 1974: 404 pages: 55 line illustrations and 40 pages of plates: 0 412 10730 9: hardback: £9.00

The subject of the solar chromosphere is central to modern developments in both solar physics and astrophysics generally, not least because modern observations suggest close links with such diverse phenomena as the solar wind and the deep-lying convection zone responsible for supergranulation.

This book forms a comprehensive discussion on the solar chromosphere and the many illustrations and tables give the reader easy access to modern quantitative data on the subject. The emphasis throughout is placed on the quiet chromosphere although a short chapter has been included on the active region.

Experimental Physics for Students

R. WHITTLE AND
J. YARWOOD

December 1973: 370 pages: numerous illustrations: 0 412 09770 2: hardback: £4.95

This textbook describes in detail about 135 experiments suitable for undergraduate students. All the experiments described have been carried out in the laboratory and in all cases the necessary theoretical background is outlined before full descriptions are given of the procedure. In many instances, typical experimental results are provided and the analysis of the significance of these results is discussed.

A complete catalogue or further information on these titles, together with a list of stockists, is available from the publishers on request.

CHAPMAN & HALL,
11 New Fetter Lane,
London EC4P 4EE

polama are indicated. Of pronghorn antelopes, there was a small *Breameryx* and two larger species, probably both *Tetrameryx*. Several finds of *Bison* sp., including a fine skull with horn-cores, are compared with known New World species. Edentates included a large armadillo (*Holmesina*), a single ossicle of a glyptodont, *Brachyostreon*, and the flattened pelvis of a ground-sloth, *Nothrotherium*.

Most of the remains came from the Middle (see below) fossiliferous horizon, perhaps datable to an interstadial of the Last Glaciation corresponding with the European Paudorf, 28,000–25,000 years BP.

H. Bunde gives topographical and geological maps marking the positions of more than 190 exposures visited, from which some 1,200 samples of loose sediments and 80 hard-rock samples were collected. Pains-taking heavy-mineral analyses of these enabled him to divide the late Tertiary to Recent deposits into four main zones, according to their dominant heavy-mineral species: Pliocene, an Mg-hornblende zone; Pleistocene, a thick Fe-hornblende zone, interrupted near its summit by pumice and ash falls with dominant olivine, from a local vent. This included the lower fossiliferous deposits. The middle fossiliferous horizon lay near the base of the succeeding hypersthene zone, to which a Wisconsin date is attributed. Above, including the uppermost fossil beds, was a zone with dominant biotite, of Late Glacial and Postglacial date. The accompanying mineral spectra make these subdivisions perfectly convincing, and on their evidence were based correlations leading to a composite stratigraphical column in the above sense.

There is a clear potential practical value of this conclusion, for correlating generally very varied and only locally occurring fine-grained strata of differing appearance, often more or less weathered or redeposited by wind or water and in no other way readily assignable to their relative positions in a sequence. It may well have important results in the archaeological field when applied to deposits containing mammalian fossils and artifacts.

G. Nobis's study of the horse teeth recognised three groups: two larger species with primitive 'zebroid' features, the first of which might be assigned to *Equus* (*Hesperohippus*) *mexicanus*, the second remaining indeterminate. A third, smaller, species resembles *Equus conversidens* Owen, an asinoid parallel with the Eurasiatic *E. hydruntinus*. The specimens in question are well illustrated in two plates.

E. W. Guenther's contribution is an exhaustive study, with numerous graphs and scatter diagrams, illustrating the

morphological and metrical characters of a collection of between 40 and 50 elephant teeth. He compares them with others found in Mexico and with the well known rich collections from Süssengborn (*P. trogontherii*) and Vogelherd (*M. primigenius*). He concludes that most represent *M. columbi*, about 10 of them *M. imperator* and only two, without provenance, *M. primigenius*. To these he adds a late, dwarf, species, hitherto undescribed, which he calls "*M. primigenius diminutivus* var. *mexicana*". The upshot is that practically all the elephants are of interstadial or interglacial forms, as the stratigraphy would suggest. The study is of methodological importance and introduces a number of new techniques for the measurement and graphical presentation of such material.

In all, this is an important publication, which should interest not only Americanists, but geologists and palaeo-anthropologists in general. The price, however, may restrict its availability to libraries only.

I. W. CORNWALL

Problems with light

Problems in Optics. By M. Rousseau and J. P. Mathieu. Translated by J. Warren Blaker. Pp. ix+366. (International Series of Monographs in Natural Philosophy Volume 52.) (Pergamon: Oxford and New York, 1973.)

THIS collection of problems in optics includes not only the classical aspects of physical optics, such as diffraction, interference, polarisation and dispersion but also electromagnetic waves, atomic and molecular spectroscopy and quantum mechanics. Although the problems set are keyed to the complementary text, *Optics* by Mathieu, in the same series, it is not really necessary to have access to this book to make good use of the present volume. The approach adopted is to set, usually three, closely related problems and to follow these by detailed solutions, each of which begins with a summary of the relevant physics required. There are many numerical computations so that the student is adequately drilled in the manipulation of units (MKSA rationalised units are used throughout) and is also given practice in estimating the orders of magnitude of the various quantities involved. The book can be fairly said to have met the two principal objectives of its authors, to teach the principles of optics and to teach the methods of problem solving. The text is well printed and the diagrams are clear and easily understood. The translation from the original French is excellent and reads well. There is good consistency in the use of symbols and constants with a convenient summary at the beginning.

With the renaissance in optics brought about by the developments in laser physics and space research, this book has come at an opportune time and should be particularly valuable to research workers in solving real problems, as well as to academic staff, who have to set examination questions, and to students who have to answer them. I have already found it invaluable in helping tutorial students to solve honours degree examination problems. To sum up, this is an excellent book and I strongly recommend it.

D. J. BRADLEY

Where the oil is

Oilfields of the World: Geology and Geography. By E. N. Tiritsoo. Pp. viii+376. (Scientific: Beaconsfield, 1973.) £8.50.

THE subtitle of this book, *Geology and Geography*, is an unduly large claim for a volume of only 376 pages. Nevertheless it is a useful and up to date reference book, the more valuable for its appearance at this critical time in the history of exploration of natural hydrocarbons.

The book begins with an introduction on the history, nature and occurrence of oilfields. Much of this is elementary, but it has the merit of defining and explaining many of the terms peculiar to the petroleum industry, definitions often not readily available to the non-specialist (for example 'naphtha', 'middle distillate', the niceties of varying reservoir type and of different ways of quoting reserves figures).

The greater part of the book is occupied by a catalogue of the world's oilfields. This is somewhat uneven in emphasis, with relatively modest space given to the Middle East in proportion to the bulk of its reserves (a tenth of the space for half the world's oil), but it has the particular merit of giving generous treatment of areas on which information is much more difficult to obtain—notably Eastern Europe, the Soviet Union and Eastern Asia. The treatment of the United States is perhaps disproportionately long (twice the space allotted to the Middle East) but the enormous number of small fields and the overall complexity would make any shorter treatment very difficult to arrange.

The main disappointment is that the treatment of geology is scanty in the extreme. The age and nature of reservoir beds is quoted for most of the oilfields described, but there are no stratigraphic columns, no structural maps, and no structural sections except for the generalised textbook diagrams in the introduction. With a great wealth of available material to draw upon in the world's literature, this seems a

major opportunity lost, and the brief descriptions included in the text do not go very far to fill the lacuna. The net effect has been the production of a highly informative geography of petroleum sources, but it is a catalogue rather than a stimulus to ideas.

Participants in the recent Royal Society symposium on "Energy in the 1980s" may be interested to see that units are given uniformly in barrels per day or barrels per annum. There is certainly merit in uniformity, and the author has successfully avoided the pitfalls of different kinds of tons and of (for example) the three different official conversion factors for "coal equivalents". Nevertheless it would have been useful to have had summary figures given in energy terms, in joules or 'Q', for those users of the book who are attempting to relate mineral oil to the statistics of other sources of world energy.

The book ends with a chapter on reserves, requirements and future supply, in which the potential for expansion of conventional sources, and the alternatives provided by tar sands and oil shales are discussed. Dr Tiritsoo echoed the warnings of Warman in the United Kingdom, Halbouty, and others in the United States, that the rate of discovery of giant oilfields (the significant contributors to the world's reserves) has fallen sharply since 1960, and—writing before the current potential crisis—he concluded by underlining the decision of Kuwait and Iraq, made in 1972, to curtail production against future requirements with the apposite quotation from the Rubaiyat:

"I often wonder what the Vintners
buy
One half so precious as the stuff
they sell".

The industry has not been devoid of people clear-sighted enough to envisage the present crisis; it is a pity that governments have not always been equally prescient.

P. E. KENT

Background to a battle

The Quantum Theory of Light. By R. Loudon. (Clarendon: Oxford; Oxford University: London, November 1973.) £7.50.

THE quantum theory of electromagnetic radiation has once again become the subject of lively discussion, thanks to the advances in the field of laser physics. These advances have made easily available sources of radiation with coherence and intensity characteristics completely different from those used by experimenters in the past and have produced new controversies over the specific application of quantisation to radiation fields.

But before it is possible to enter the quite heated battles surrounding this subject, it is necessary to obtain a grounding in the basic theoretical concepts involved in the discussion of the interaction of radiation with matter. Dr Loudon's book provides an excellent beginning to such a grounding, at a level suitable for graduate students, or good finals-year undergraduates.

Beginning with a review of classical radiation theory as applied to emission and absorption processes, the classical theory of optical coherence is briefly discussed. Quantisation of the radiation field is introduced, together with the properties of photon phase and number operators. This leads to a discussion of photon absorption and emission in terms of quantised field theory and a general discussion of photon optics including a brief section on laser theory and nonlinear optics.

To briefly single out only two chapters: the discussion of classical coherence theory is very well done and the notation carefully chosen so that an easy transition to the quantised approach can be made. Similarly, the discussion of photon optics provides an excellent introduction to the subject.

The general production of the book is excellent. The salting of the text with problems, which might be thought inappropriate at this level, seems an excellent idea and the references at the end of each chapter are comprehensive.

Although I have definite reservations on the blanket use of quantum field theory in optics, this book can be highly recommended to students in the field.

D. G. C. JONES

Unimolecular reactions

Theory of Unimolecular Reactions. By Wendell Forst. Pp. xv+445. (Physical Chemistry: vol. 30.) (Academic (Harcourt Brace Jovanovich): New York and London, November 1973.) \$29.50.

ANYBODY who has had a more than superficial interest in the theory of unimolecular reactions will be familiar with the names of Wendell Forst and O. K. Rice, who contributes the foreword. The latter's encouragement and stimulus to many research workers in this field, including Wendell Forst, should not pass without mention.

The volume is divided into two parts, the first of which deals with the general concept of the unimolecular process, the definition of such basic quantities as average rate and the basic assumptions involved. Slater theory is mentioned only in passing and to place its assumptions in correct perspective. There follows a discussion of intramolecular energy transfer and the assumption of energy randomisation. Chapter 4 considers the statistical cal-

ulation of the unimolecular rate with respect to phase space in both classical and quantum-mechanical formats; pertinent degrees of freedom are thoroughly discussed, due attention being drawn to the necessary restrictions implied by conservation theorems. As might be anticipated, a lengthy and excellent chapter on energy-level densities is included and should enable the reader to utilise the various approaches in actual calculations.

Part 2 divides itself naturally, through the average energy of the excited species, into a discussion of thermal reactions, chemical activation systems (including photoactivation) and, most welcome, a detailed consideration of the fragmentation of highly excited molecules and ions. These make excellent reading indeed, and the detailed analysis of rotational effects, where appropriate, is certainly worthy of comment.

References are given at the end of each chapter and are both extensive and up to date; a glossary of the symbols used, together with their clear definitions (always welcome in this field), is given as well as an author and subject index.

The author rightly appeals in an appendix, for the more extensive use of the results of the steepest descents method of calculating energy-level densities. A simple basic programme is given as further encouragement.

I found my task of reviewing this book both rewarding and enjoyable, and even at a price in excess of twelve pounds, no one with any pretensions to interest in this field can afford to be without a copy.

W. J. ORVILLE-THOMAS

Arctic circle

Arctic Geology. (Proceedings of the Second International Symposium on Arctic Geology, held February 1-4, 1971 at San Francisco.) Edited by Max G. Pitcher. Pp. xvii+747. (American Association of Petroleum Geologists: Tulsa, Oklahoma, 1973.) \$30.

THERE is a certain scale of geological phenomenon that may suddenly come into focus when the appropriate mixture of specialists come together at a symposium. The formula works particularly well when the solution of a geological problem requires knowledge of the geology of a number of countries. This book contains some seventy papers read at a symposium on Arctic geology held early in 1971. To my mind the result is particularly rewarding in two ways. The specialist will find discussions of the geology of Alaska, of Arctic Canada and the Soviet Union, of the Arctic ocean, the North Atlantic and the North-

ern arc of the circum-Pacific fold belts together with first class reviews of various stages in their geological history.

But above this and of more importance to the science as a whole the symposium succeeded in illuminating the geology and the economic potential of the succession of sedimentary basins which overlie the Precambrian blocks clustered around the Arctic Ocean. By the end of the century a quarter of the world's production of hydrocarbons may come from the Arctic. The ring of Precambrian blocks which underlie Canada, Greenland, Northern Europe, north-west and north-east Siberia have separated and come together in a complicated fashion during the past 500 million years. The mountains of Scotland and Scandinavia, the Appalachians, the Urals, the island arcs and the mountain chains around the northern Pacific and the North Atlantic itself are direct results of these movements. Their history is traced out in this book, as is the history of the sedimentary basins which are so important as potential or actual sources of petroleum. This book can be recommended as providing an excellent synthesis of a region likely to become of ever increasing importance in the remainder of this century.

JOHN SUTTON

Impurities have virtues

Deep Impurities in Semiconductors. By A. G. Milnes. Pp. xviii+526. (Wiley-Interscience: New York and London, 1973.) n.p.

STUDIES of the electrical properties of semiconductor crystals containing impurities fall into at least two distinct categories. The first is concerned with the determination of the position and nature of localised electronic states due to specific impurities, and perhaps the accounting for these properties in terms of theoretical models. The second category relates to the electrical behaviour of such material under non-equilibrium conditions and to the properties of junction structures. It is important to recognise that work in these two areas is in general carried out by different groups of research workers and consequently the bridge between them is often quite tenuous.

The present volume aims to be complete in this context, although analyses of defects produced by irradiation damage are explicitly omitted. The theory of localised energy levels is discussed briefly in chapter 1, which is followed by reviews of data for silicon, germanium, gallium arsenide, gallium phosphide and other III-V compound crystals in chapters 2 and 3. The latter chapters read very much as a catalogue,

but are nevertheless valuable because of the large number of references cited. There is then a marked change in style in chapters 4, 5 and 6, which deal with theories of steady state statistics of deep impurities, trapping and recombination. Apart from a few examples, of which gold doped silicon is prominent, the deep levels are not considered in relation to particular systems. The emphasis becomes more practical in the following chapters with discussions of photoconductivity, recombination and trapping in junctions, thermally stimulated currents and techniques for measuring lifetimes.

The contents of the remaining four chapters are of considerably more interest. It is shown in some detail how the presence of deep impurities can lead to a negative differential resistance, and oscillatory effects are discussed at length. The relevance of the final chapter on conductivity by hopping processes to deep impurities is not too clear, as is pointed out by the author in the very final paragraph.

An overall impression gained from this book is that the difficulties referred to in the opening paragraph of this review have not yet been overcome, although of course this in no way reflects adversely on the text. A second impression is that the author has given prominence to the beneficial aspects of deep impurities, citing the value of infrared detectors and possible future uses of oscillatory phenomena. Adverse effects such as the quenching of luminescence, reduction in carrier mobilities and lifetimes would be regarded by many as being equally if not more important. Finally it should be noted that the very large bibliography containing nearly 1,900 items should be invaluable to workers in this field.

R. C. NEWMAN

Ancient climates studied

Climatic Fluctuations of the Ice Age. By Burekhard Frenzel. Translated from the German by A. E. M. Nairn. Pp. xxiv+306. (Case Western Reserve University: Cleveland and London, November 1973.) \$22.50.

THE world's climate is not only changing, but changing on a timescale rapid enough to affect present day economic planning. Interest is growing in the problems of climatic change in general, and in such spectacular examples as ice ages in particular. So the arrival of this translation of Frenzel's 1967 German language study of the most recent ice age is particularly well timed.

The book concentrates upon facts, rather than hypotheses to explain the factual variations, and thus provides a useful reference for anyone wishing to



PINGOS in East Greenland. Squeezed between the permafrost layer below and the winter freezing layer above, the water-bearing horizon in some Arctic regions can be subjected to pressures of 50 atmospheres. This causes hillocks to form, up to 40 m high and 100 to 200 m in diameter; when the ice core of the pingo eventually melts, a crater-like depression is left, and these depressions may remain as a visible record of a period of severe climate.

jump off into the unknown with their own hypotheses. Climatic fluctuations during the Pleistocene undoubtedly have their origins in the same changes in the global pattern of circulation, or of input of solar radiation, that cause the climate to change today—but of course, it is not yet clear exactly what those changes are. This well planned and thorough discussion of the methods by which ancient climates can be studied, and what comes out of those studies, will be particularly useful to those people from many disciplines who are turning part, at least, of their attention to these questions; not least among the useful features of the book is the comprehensive bibliography.

JOHN GRIBBIN

How many worlds?

The Many-Worlds Interpretation of Quantum Mechanics. Edited by Bryce S. DeWitt and Neill Graham. Pp. viii+252. (Princeton University: Princeton, November 1973.) \$12.50.

THE 'many-worlds' interpretation is surely the most exotic product of the endless struggle to understand quantum mechanics. To avoid any suggestion of the infamous 'reduction of the wave packet' it is supposed that not only does the wave function not reduce, during measurement, but that physical reality multiplies. On each occasion not just one but all possible measurement results are realised! To accommodate them all an increasingly multiple world is required, one 'branch world' for each

possible sequence of results. It is supposed that, in each branch, objects (including people) interact with (and are conscious of) others in the same branch only. So we are not aware of the many worlds that we do not inhabit; the people, often very very like us, living in those other worlds, are other people.

If you have not personally found the time to grapple seriously with the puzzles of quantum mechanics it can hardly be intelligible to you that physicists might be driven to such lengths. And even if you have found the time, you may wonder in what way such a conception is superior to the de Broglie-Bohm pilot wave theory; there also the wave never reduces, but only one of the many possible physical configurations is realised, and evolves in time in a way consistent with quantum mechanical probabilities. My opinion is that the pilot wave provides a simpler and more economical solution of the problems motivating both approaches, and is quite superior in requiring neither any arbitrary classification of physical processes into 'measurements' and others, nor any arbitrary division of the world into 'systems' and 'apparatus'. I have to add, however, that my understanding of the pilot wave theory was much enhanced by some aspects of the 'many world' picture, in particular its emphasis on memory states. Moreover it is the very clarity and precision of the pilot wave theory which puts in prominence the particular difficulty concerning locality. But this is not the place to develop that theme.

The book under review is mainly a collection of preprints: the original 1957 paper of Everett, a contemporary assessment of it by Wheeler, later expositions by de Witt (1969 and 1970), and a later rediscovery of similar ideas about correlations in memory states by Cooper and Van Vechten (1969). Cooper and Van Vechten explicitly invoke 'irreversibility' to distinguish some physical processes from others as 'measurements', so exhibiting more prominently a basic vagueness of the approach. They also explicitly dissociate themselves, in a footnote added in 1972, from the many worlds concept; they see no reason for more than one of all the possible worlds actually to 'come to pass'. This puts them still closer to de Broglie and Bohm, but even they do not recognise this affinity.

In addition there are two hitherto unpublished papers. One is an alternative account by Everett himself, written apparently before the well known paper mentioned above. Although it does not add anything (it seems to me) on central points it contains some interesting auxiliary material, including in particular a discussion of various measures of uncertainty, and some comments on other approaches to quantum mechan-

ics. Here the de Broglie-Bohm picture is mentioned; Everett thinks his own picture is simpler. The other new paper is by Neill Graham. He is concerned about the arbitrariness of the measure (actually the quantum mechanical norm) invoked to weight the various branch worlds. He argues that with sufficiently complicated (ergodic) apparatus simple equal weighting of different branches is practically equivalent. In the form actually given the argument supposes the multiple world divided into discrete branches, classified by the discrete eigenvalues of some privileged 'observables'. That suitable operators with discrete eigenvalues can be considered in the neat world of academic quantum mechanical measurement theory is clear. The messy real world, in which each piece of measuring equipment is immersed in a particle and radiation bath from which it is only vaguely separated (on the microscopic scale) may be another problem.

The search for a satisfactory interpretation of quantum mechanics is likely to continue for a long time among many, rightly or wrongly, unsatisfied people. The many worlds interpretation, its tenability or untenability, and the sidelights it throws on other schemes, will continue to be discussed. For participants in that discussion this will be a useful book to have.

J. S. BELL

Quantum textbook

Fields and Particles: An Introduction to Electromagnetic Wave Phenomena and Quantum Physics. By Francis Bitter and Heinrich A. Medicus. Pp. xiii+688. (American Elsevier: New York, London and Amsterdam, 1973.) Dfl. 52; \$20.

THIS is an ambitious book which takes the reader from electromagnetism through a wide variety of wave phenomena to a discussion of the symmetries of elementary particles. For so large a book the price is very reasonable, and at its best its merits are sufficient for the buyer to feel he has got his money's worth. Nevertheless there are notable imperfections which cause me to hesitate before recommending it as the basis of a major course at, say, first or second year level in an English university.

To begin with a few minor examples, as typical of the rather numerous blemishes to be eliminated in the next edition: it is implied (page 199) that as a wave packet spreads by dispersion, the frequency range in its composition gets less; the discussion of Poynting's vector (page 627) involves a mathematical oversimplification which carries the corollary that all lines of force are straight; the phase-amplitude diagram

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British Book News

Statistical Tables for Biological, Agricultural & Medical Research

Sixth Edition
Fisher and Yates

This book of statistical tables, first published in 1938 by Oliver & Boyd, is now being reprinted by Longman. This is the Sixth Edition (1963) with minor corrections. It has long been regarded as a standard work essential for all statisticians and biometricians, especially those concerned with investigations on highly variable material. £2.50



Longman
1724 - 1974

(page 90), so essential for an appreciation of diffraction at this level (and for that matter at a much higher level), is thrown in far too peremptorily for a student whose experience of complex numbers is as rudimentary as seems to be assumed; Fourier analysis (page 61) is left rather as a mystery, when a few pictures could have brought the mathematics to life.

But even with these faults corrected, I should still have doubts, and not only those of a partisan of the solid state who wonders whether it is still so obviously the overriding goal of physics to press on single-mindedly in the pursuit of the fundamental laws. It is not just this, but a feeling (of which some of the specific points just cited are evidence) that the book does not encourage the student to grow as he learns. There are some comparatively elementary texts (Born's *Atomic Physics* springs to mind) to which one can turn again and again, after a spell of more advanced work, to find that the heart of the matter is there; the learner's difficulties are appreciated and overcome without condescension. Such books the serious student will cherish all his life, but this I fear is not one of them. If he comes back to it he will feel that he was talked down to just a little, that the book explained to him little more than what it set out to explain, without any of the shafts of illumination that a great master can give—cannot but give—along the road. In short, it has the makings of a sound textbook; it could not be called, in the highest sense, a work of scholarship. And this a pity, for our best students deserve the best we can offer.

A. B. PIPPARD

Spinning electrons

Electron Spin Resonance: Theory and Applications. By N. M. Atherton. Pp. x + 438. (Ellis Horwood, distributed by Wiley: Chichester; Halsted (Wiley): New York, November 1973.) £11.50.

THE theory and techniques of electron spin resonance have now been developed for almost thirty years, and yet the subject still has its growth points, and is still attracting large numbers of research students. The author of this text has seen a real need to provide a basic reference text for new (and existing) workers in this field. Most books on this subject require frequent recourse to other sources, but in this case most aspects of the theory are developed from a sufficiently elementary level to make the text particularly useful to postgraduate physicists and chemists. In that it deals essentially with the theoretical background to the subject, the text forms a useful companion to other recent publications on experi-

mental techniques cited in the bibliography.

The first two chapters of the book deal essentially with introductory aspects of the subject, and form a sound basis from which the subject can be developed. They are followed by two chapters dealing principally with most aspects of the ESR spectroscopy of radicals in solution and trapped in solids. Chapters 6 and 8 deal with the theory of transition metal complexes and with the basic theory of the g -tensor and are followed by two comprehensive chapters on relaxation and lineshape theories. The interpretations of experimental parameters such as g values and hyperfine splittings for simple systems are developed extensively. The sections concerned primarily with transition metal ions tend to stand out in contrast to other sections in that the theory is not developed from quite the same basic level as in the earlier chapters: an inevitable consequence when trying to cover such an extensive subject in so short a space. The problem is helped however, by an excellent bibliography. The final chapters are concerned with two particular aspects of the subject: double resonance and gas phase electron resonance.

The whole range of material covered by the text relies considerably on a basic understanding of quantum mechanics. Though the level of knowledge required is beyond many undergraduate physics and chemistry courses it would require very little effort for most postgraduate students to achieve the necessary standard. The text might have benefited from a brief appendix outlining the principal techniques required such as elementary matrix methods, perturbation theory and some aspects of angular momentum theory. The majority of the topics are developed from an elementary level but unfortunately, although perhaps inevitably, the discussion ceases before many of the interesting examples. Many of the topics treated could have benefited from a few more illustrative numerical examples.

In conclusion I should say that the text forms a very worthwhile contribution which will certainly be a standard requirement for most laboratories working in this field. Many workers in electron resonance spectroscopy have long felt the need for a work of this kind, and I hope that the publishers will consider producing a cheaper paperback edition. The author has undoubtedly achieved his aim described in the preface, namely, to produce a book which will prepare people to read the electron spin resonance literature and that would be useful for a number of years.

E. F. SLADE

BIOLOGICAL SCIENCES

Promoting plants

Phytochemistry: the Process and Products of Photosynthesis. Edited by L. P. Miller. Vol. 1, pp. xiv+410; vol. 2, pp. xiv+445. (Van Nostrand Reinhold: New York and London, June 1973.) Vol. 1, £11.25; vol. 2, £12.25.

The Biochemistry of Green Plants. By D. W. Krogmann. Pp. xiv+239. (Prentice-Hall: Englewood Cliffs, October 1973.) £11.95.

PLANT biochemistry has always been a Cinderella subject, and a Cinderella who has not yet met her fairy godmother. Due principally to the attitude of mind that M. D. Kamen has referred to as "mammalian chauvinism", the study of the biochemistry of plant processes has to date been an undeservedly neglected field. Even the rawest biochemistry undergraduate appreciates that the fixation of atmospheric CO₂ by green plants utilising solar energy is the most important chemical process in the biosphere; nevertheless as he pursues his biochemical career, he gradually becomes indoctrinated into the view that those processes which occur in animal cells are of paramount importance and that the study of plant processes is not a suitable field in which to carve a career.

Fortunately, there are signs that this trend is due to be reversed. The consuming requirement for increased global food supplies is bound ultimately to direct attention towards the mechanisms of plant growth and productivity, which surely can only be properly understood at the biochemical level. Both of the books under review attempt in their own completely different way to act as fairy godmothers for plant biochemistry, one by selecting for detailed treatment the currently most fashionable topics within the subject, and the other by dealing exhaustively with the peculiar ability of plants to synthesise substances of fascination for the organic chemist.

In his selective textbook Dr D. W. Krogmann has attempted to show, in terms that an undergraduate can assimilate, the excitement that investigating plant biochemistry can bring. It does not attempt to deal with secondary product biosynthesis, at which plants are adept, but does give intensive coverage of a range of processes which either only occur in plants, or are particularly well illustrated by plants. These include hexose breakdown, nitrate and sulphate reduction, photosynthesis, cell wall biochemistry, fat metabolism, chloroplast development, photomorphogenesis and hormone action. It is undeniable that there is a substantial gap

in the literature which this book attempts to plug. The treatment, however, is rather patchy. The detailed coverage of photosynthesis, hexose metabolism, cell wall synthesis and lipid metabolism is very complete and would be of great assistance to students of plant biochemistry. On the other hand, the book falls down a little in the area of control of metabolism by light and hormones—these, however, are immense subjects in their own right and perhaps the superficial coverage presented here would be considered sufficient by most undergraduates. In any case, the book is a welcome addition to the available literature, not least because it is written in an entertainingly informal style (including verses from Ogden Nash) and yet fearlessly challenges the view that the only really interesting biochemical processes go on in *Escherichia coli* and *H. sapiens* var. HeLa.

The opposite approach is taken by Dr L. P. Miller who has undertaken to edit a three-volume treatise (only the first two volumes of which are yet available for review) on phytochemistry. In contrast with Dr Krogmann's book, the treatise is substance-oriented rather than process-oriented. This will undoubtedly ensure its popularity with organic and natural product chemists, but will probably mitigate against its purchase by genuine plant biochemists of which in any case there are very few. The first volume deals with photosynthesis, photosynthetic pigments and photosynthetic products, namely the various forms of carbohydrates. The second volume covers the various organic metabolites synthesised by plants including amino acids, proteins, purines and pyrimidines, alkaloids, steroids and flavonoids. The standard of coverage, both in comprehensiveness and depth of detail is consistently of the highest order, as would be expected of the very distinguished contributors to both volumes.

The treatise is clearly not intended as an undergraduate text, however, and it is likely to appeal principally to the research worker and university teacher with specialised interests in natural products. The very scale of the production emphasises the enormous amount of purely chemical information that is available on the substances present in green plants and this treatise should help to accelerate the exploitation of plants for the synthesis of rare and valuable chemotherapeutic agents. As a source book, this treatise should be extremely valuable on the shelves of any biochemical library. Neither of these books will, on their own, pull plant biochemistry out of the doldrums, but they

both serve admirably to emphasise the unique and critically important role of plants in the living world. They should be required reading in all biology and biochemistry departments.

H. SMITH

Lithium therapy

Lithium: Its Role in Psychiatric Research and Treatment. Edited by Samuel Gershon and Baron Shopsin. Pp. xi+358. (Plenum: New York and London, 1973.) \$22.50.

THE use of lithium for the treatment and prevention of recurrent mental illness has been the subject of a remarkably vigorous debate. This book presents an intriguing mixture of chapters from clinical psychiatrists using lithium therapy and also from various groups who are pursuing some of the many possible lines of enquiry into the mechanism whereby a simple metal ion can restore abnormal mood to normal and actually prevent future severe mood changes. It would perhaps have been interesting if one chapter in the book had been written by a representative of the English group of psychiatrists who have so forcibly questioned some of the claims made for lithium therapy.

The careful and detailed research by Danish workers is clearly explained by

Proboscis



ADULT female Günther's dik-dik, from *The Ruminant Stomach: Stomach Structure and Feeding Habits of East African Game Ruminants* by R. R. Hofmann. (East African Monographs in Biology vol. 2.) (East African Literature Bureau: Nairobi, 1973.) Boards £10.60; paper £6.50.

Dr Mogen Schou in chapters 10 and 14. Dr Schou of course, has done much to promote this treatment by his patient advocacy of the real and unique clinical benefits of lithium therapy in the affective disorders. I particularly liked Dr Schou's suggestions that clinicians should record dosage in millimoles lithium, and that drug suppliers should indicate the lithium content of their preparations in the same units. His plea that research reports should state the timing of blood taken for lithium analysis in relation to the last oral dosage of lithium salt is also worthy of mention.

As a clinical chemist I would have wanted some extension of the remarks in chapter 14, page 292 on the need for proper control of serum lithium analysis—and in this context cannot resist asking for more details of the method which allows Drs Gershon and Shopsin in chapter 7, page 133 to quote serum lithium concentrations to three decimal places. (This in no way detracts from a useful account of lithium toxicity.)

The opening and closing chapters of the book, by Drs N. S. Kline and R. R. Fieve are especially stimulating and outspoken. I was interested, for example, to be reminded by Dr Kline that lithium has a real historical claim to be among the first of the "psychic energisers" and to read that the very cheapness and availability of lithium salts has made drug houses reluctant to market preparations which may compete with other more complex (and profitable) drugs.

In due course it can be expected that lithium will be superseded by more effective and less toxic substances once the basis of its action on mood is clearly understood. Until that rather distant time comes this volume will be required reading for all of those who wish to improve the treatment of patients suffering severe mood disturbance, by the use of well controlled lithium therapy.

G. S. FELL

Runaway chromosomes

Chromosomes in Human Cancer. By J. Cervenka and L. Koulischer. Pp. viii + 203. (Thomas: Springfield, Ill., July 1973.) \$13.75.

THE stated purpose of this book is to "gather all available information on chromosomes in human tumours with the fervent hope that they might be used as a source of understanding of the seemingly disordered chromosomal constitution in human cancer cells and of the few rules which may be derived from these data". The authors have definitely succeeded in the Sisyphean task of gathering the information. The book will be a precious source for the quick identification of sources. Is it pre-

sumptuous for one who has no clinical qualification to suggest that the fervent hope has not been fulfilled?

The few rules which emerge are commonplaces of the dynamics of cell populations, and indeed of populations in general. These rules derive from first principles of evolutionary genetics (including cytogenetics) and have been experimentally confirmed with unicellular organisms and with cultured somatic cells and transplants of higher organisms including man.

The value of this book does not lie in illuminating the chromosomal mess in tumours. (Incidentally, is enough known about chromosomal variation in normal somatic tissues, especially embryonic?) It lies in the many tables—at least one for each of the major forms of human cancer—which summarises very clearly the published cases in which chromosomal analysis was performed. For this service those engaged in research on tumours, both clinical and experimental, should be grateful to the authors.

G. PONTECORVO

Life is inorganic too

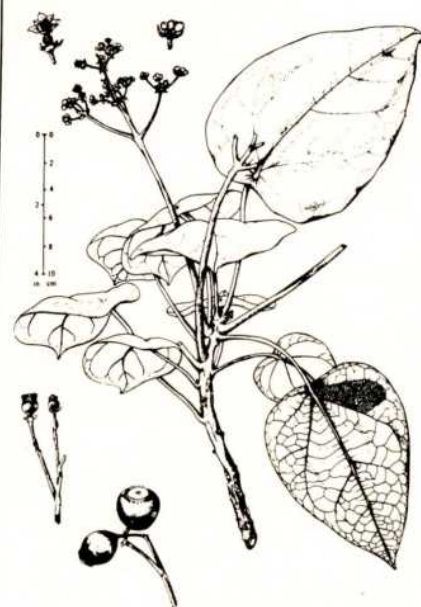
Inorganic Biochemistry. Edited by Gunther L. Eichorn. Vol. 1 pp. xxiv + 1-608; vol. 2 pp. xi + 609-1263. (Elsevier: Amsterdam, London and New York, 1973.) Dfl. 300; \$105.30.

THESE two volumes do not form a book. Rather they hold together thirty-four booklets running from the necessary inorganic chemistry for the understanding of biological roles of metal ions to the description of the metal-containing biological systems themselves. All the important roles of metals are described but most of the booklets are written by an authority largely for other authorities and in a style which precludes a general reader from getting past the first few pages. Many of the chapters are not therefore readable at a sitting and they could as well have appeared in a review journal where the objective is extensive reference rather than intelligent discussion. In fact it is frequently the case that the biological significance of the materials under discussion is lost among the mass of detail on absorption spectra, EPR signals and Mössbauer data. Striking exceptions are the chapters of Nielsens on ion carriers, and by Spiro on phosphate transfer.

I feel that the title of this book should have been 'The Properties of Inorganic Materials found in Biological Systems' for the stress is too far from biological function. The question then arises: is this a book on inorganic biochemistry?

For many years most scientists have looked upon living systems as a growing and self-reproducing organic matrix.

Tropical tree



LEAVES, fruit and flowers of *Hernandia nymphaeifolia*, so called because of the similarity of its leaves to those of the water lily *Nymphaea*. This small tree grows on sea coasts in East Africa, southern Asia and the south Pacific. From *Tree Flora of Malaya* vol. 2, edited by T. C. Whitman for the Forest Department of Malaysia. (Longman: Essex, July 1973.) £12.50.

Such a view is a natural development from the separation of the animal and vegetable from the mineral kingdoms. The distinction, perpetuated in the educational divisions of inorganic and organic chemistry has forced inorganic chemistry more and more toward physical sciences and organic chemistry toward life sciences. Today one knows that this distinction, seen in almost every chemistry journal and in most chemistry courses in schools and universities is wrong-headed. Life has evolved from inorganic materials (generating organic chemistry as it went) and in that evolution has incorporated every facet of inorganic chemistry which was profitable to it. This book clearly proves this point. The only reason for using a title such as *Inorganic Biochemistry* is to get away from the implied title 'Organic Biochemistry' every time an inorganic chemist reads 'Biochemistry'. In other words Avis quite rightly wants a share of Hertz's business: car renting, and the study of biology by chemists, is not a monopoly activity. What then is the inorganic part of biology?

The roles of inorganic materials can readily be separated into the functions of bulk structural units, such as shells and bones, control elements, such as energy stores in concentration gradients, and catalyst centres. What feel does this

book give of these different biological topics?

The great structural role of silica, calcium carbonate, and calcium phosphates is not described. The nature of the nerve pulse, of the triggering of muscle contraction, of hormone release, of coagulation, of almost what you will as far as (inorganic) control over action is concerned receives scant mention with one notable exception. The editor has made it his speciality to deal with the interaction of metal cations with the polymers of translation and transcription, RNA and DNA. Yet even in his very interesting chapters he falls into the inorganic trap. He becomes enmeshed in a net of spectroscopic read-out. In fact I surmise that the reason this book omits two of the three fields of inorganic biochemistry, solid state structures and control, is that this chemistry belongs to the elements sodium, potassium, magnesium and calcium. The inorganic chemistry of these elements is taught no more because there are no spectra to tease the mind. Out of sight (spectroscopy) out of mind, is true for inorganic biochemistry and for many other aspects of inorganic chemistry today.

The third function of inorganic elements in biology is catalysis. Catalysis is carried out by transition metal ions or by ions such as zinc which can be replaced by these highly spectroscopic cations. The apparatus of the physical chemist can be tuned in. Each of the very good chapters on iron, cobalt, manganese, copper and zinc systems, all in a variety of forms, shows very well how to examine the active site but, while using these exciting tools, I miss the answers to the questions: Why has biology finished up with a few metals, in a few sites? Why zinc for acid catalysis and not copper? In fact I find myself asking: Who says that the role of these metal ions is as catalysts? Perhaps, through their special stereochemistry, they just generate curious organic chemistry in the active site region.

Is the role of the inorganic component too muddled with the role of the organic to be extractable? Who says ferredoxins are just electron-transfer proteins? I could be persuaded that they are really just electron traps and storage units. Their design is associated with the establishment of an electron at a redox potential close to that of the hydrogen potential but such that they do not blow off hydrogen. If my points are relevant the problem of building inorganic model systems is not what it seems to be for the overriding consideration is a control over thermodynamic not spectroscopic properties and sometimes even a prevention of a reaction. Biology has to store electrons (and elements) and it has to control activity as

well as to catalyse reactions. By looking at spectroscopic signals too closely there is the danger that the purpose of biology is lost.

Clearly this book is extremely valuable for the specialist, but when it appears on library shelves (it will not appear anywhere else at \$ 105) it must be catalogued under Inorganic and not Organic Chemistry using the real meanings of words.

R. J. P. WILLIAMS

Primate morphology

Form and Pattern in Human Evolution: Some Mathematical, Physical, and Engineering Approaches. By Charles Oxnard. Pp. ix+218. (University of Chicago: Chicago and London, 1973.) £5.65.

THE scientific basis for primate morphological and evolutionary studies has widened dramatically in the past two decades. This book describes both the more established of these new techniques as well as others whose robustness and application are still being investigated.

Charles Oxnard is the doyen of this rapidly expanding 'technological' approach to primate morphology. It is much to his credit, and to the credit of the schools at Birmingham and Chicago, that hypotheses in studies of both extant and fossil primates are now being tested by experiment and analysis. The examples he gives of the application of these techniques are almost exclusively of work by these two schools and a more cynical reviewer than I might be justified in suggesting that a comprehensive collection of Oxnard's reprints would save the cost of the book!

This somewhat parochial approach has led to two significant omissions. The first is the lack of any assessment of the biomechanical analyses of extant and fossil primate limb material that have been published recently by Preuschoft. Many workers have been waiting for someone of Oxnard's competence in this field to assess the relative proportion of wheat and chaff that is contained in this particular technique.

A second, more important, omission is that allometry is not seriously considered. A significant part of multivariate morphometrics has been concerned with the identification of the effects of size on shape. A review and discussion of the role of factor and principal component analysis in such studies and of the work of Jolicoeur, Gould and Reyment would have been welcome.

Apart from two introductory chapters, and a useful chapter devoted to simple testing procedures, the book is devoted to explanations and the applications of multivariate, group finding, stress and optical data analysis. With-

out exception these expositions make the most complicated methods plain. The chapters on multivariate morphometrics and optical data analysis are particularly good. It is a shame that in the former chapter, the author has been tempted into making over-zealous phylogenetic interpretations of the results of the shoulder girdle analysis. The new axes generated through the universe of data on the shoulder do indeed seem to have a functional, but not necessarily evolutionary, significance. It would be a pity if this classic study of function and morphology is affected by the fallacy that the proximity and sequence of extant groups is a guide to their phylogenetic relationships. This same fallacy has bedevilled so many molecular biological phylogenetic studies.

A point of minor confusion is the use of factor analysis as an umbrella term to include principal components analysis; there are of course differences between the two techniques.

These criticisms do not seriously detract from any of the books usefulness; I learned much from reading it. Students and research workers would be well advised to buy and read it. Its careful production and accuracy are a credit to the author.

There is a danger that these increasingly complex methods will separate still further the 'experimental' primate morphologists from those who study hominid fossils and evolution. A few people try to do both and it is more important than ever to see to it that further polarisation of these two groups does not take place.

BERNARD WOOD

Pin down an attitude

Attitudes and Their Measurement. By Nigel Lemon. Pp. vii+294. (Batsford: London; Halsted: New York, December 1973.) £4.20.

THERE are two schools of thought on the usefulness of the concept of 'attitude' in the social sciences in general and in social psychology in particular. School A, to which Dr Lemon belongs, treats 'attitude' very seriously and regards it as "social psychology's central problem", its study being specially important when it is resistant to change and when it clashes with 'social structural' factors. School B, to which my sympathies incline, is described by Dr Lemon as "a substantial group who would deny the need for such a term and who would argue that attitude adds nothing to existing explanations of conduct in social structural terms".

The attempt to measure attitude was initiated by L. L. Thurstone in 1929, although as an explanatory device, it had been employed, in 1918, by W. I.

Thomas and F. Znaniecki; and it was pushed into the limelight by G. W. Allport's studies of personality in 1935. The passage of four decades since then, and the hundreds of papers on the subject cited by Dr Lemon, leave the sceptical reader with the suspicion that there is not a great deal to show for all this effort. Dr Lemon does not turn a blind eye to the critics, and he is acutely aware of what he calls "the widening gulf between the increasing sophistication of measurement and the relatively crude conceptualisation on which it is based". He treats attitude as an inference from observations, a latent process, a so-called "intervening variable". It is not, in his view, merely a descriptive expression to be defined as "the probability of occurrence of defined behaviour in a definite situation".

Whatever the explanatory merits of the concept, it must be conceded that Dr Lemon has taken pains with this work. He has immersed himself in the literature, and mastered a wide range of techniques of 'measurement'. He deals with the underlying logic of the subject, with self-administered inventories, with indirect assessment procedures, with the structure of attitudes, and with models of scaling stimuli and responses.

While praising the author's commendable skill in cogent presentation, I nevertheless still wonder how he has succeeded in allaying his own misgivings as to "crude conceptualisation". And the sceptic, whose reservations are not dispelled by a display of technical virtuosity, will continue to seek more convincing explanations of social behaviour.

JOHN COHEN

Enzymes attached

Immobilized Enzymes. By O. R. Zaborsky. Pp. 175. (CRC Press: Cleveland, 1973.) \$26.50.

DURING the past decade, many hundreds of scientific papers and tens of reviews, some good, others bad, have been published on immobilised enzymes. Here for the first time is a comprehensive book giving a reasonably balanced and objective survey of the subject.

Much of the published work has been concerned with immobilisation techniques and this is reflected in this book, almost two thirds of which are devoted to chapters describing the various techniques for immobilisation: covalent attachment, intermolecular cross-linking, adsorption, entrapment, microencapsulation and containment by semipermeable membranes. In each of these chapters, many individual methods are given, and the properties of the resulting products are described. The remaining chapters are on enzyme reactors, applications and related topics.

The scientific literature, including patents, is covered in detail up till the end of 1971. With so many scientific publications on immobilised enzymes appearing in the last two years, it is inevitable that certain aspects, for instance treatments of diffusional limitation and reactor operation, are already out of date. Lists of more recent literature are, however, available from the Corning Glass Company, USA. The chapter on applications, both industrial and analytical, inevitably is rather short of data, and as the author points out is not a true reflection of the work that has been done in industrial laboratories which in some cases is far ahead of anything published.

Nevertheless, this is an excellent book and is recommended as a useful introduction and guide for anyone wishing to acquaint himself with immobilised enzymes.

M. D. LILLY

Blue-green biology

The Biology of Blue-Green Algae. Edited by N. G. Carr and B. A. Whitton. Pp. x+676. (Blackwell Scientific: London; University of California: Berkeley, 1973.) £13.50.

The Blue-Green Algae. By G. E. Fogg, W. D. P. Stewart, P. Fay and A. E. Walsby. Pp. ii+459. (Academic: London and New York, November 1973.) £8.50; \$24.

IN times past it was usual to seek specialised information about the 'biology' of a group of organisms from ancillary sections in a major work on taxonomy, structure and reproduction. The algae were no exception, but the growing number and specialisation of biological disciplines has led to greater dependence upon reviews and occasional multi-author monographs or compendia with emphasis upon physiology, biochemistry, ecology, genetics and fine structure. The first book is a good example of the latter: with 26 contributors it has a pleasantly international flavour. In this respect, and in size and coverage, it differs from the only previously comparable work, edited by Federov and Telitchenko and published (1964) in Russian under the same title.

The 25 chapters, and 4 appendices (on cultures), are often written with considerable individuality and personal outlook—the fascinating contribution on ecology in hot springs by Castenholz is a notable example. As the editors admit, the coverage of the entire subject is uneven; some important fields (such as mineral nutrition, kinetics of growth and photosynthesis) receive only passing mention, although some specialised topics are treated in detail. The single bibliography and subject plus organism indices are valuable features, but the

book is basically a collection of independent, authoritative reviews. It constitutes an excellent and readable source of information on very many topics of the relevant biology: for a really integrated account, the reader must turn elsewhere.

He could not do better than to read the second book, *The Blue-green Algae*. Although written by four authors, their contributions merge into an organised pattern of topics and coherent style wherein preferences of individuals can only be recognised by the *cognoscenti*. The brief introduction should stimulate, after which a course is steered through three main phases of structure, physiology and ecology. An early chapter on general form and structure will be the more familiar classical area, and an essential foundation, after which deeper and more recently charted waters are traversed. First appears fine structure, and then aspects of cell physiology. Overall physiology is expressed in a chapter on culture, nutrition and growth, after which follow aspects of metabolism (photosynthesis and chemosynthesis, heterotrophy and respiration and nitrogen metabolism). Structural aspects again come to the fore briefly in an account of reproduction and cellular differentiation, but the rest of the book deals extensively with external relations—in ecology (freshwater, marine and terrestrial), pathogens and symbiotic associations. The exposition is clear and authoritative, the illustrations and figures numerous and helpful, the bibliography excellent for both classic and modern work. In the book's 'connective tissue', linking intensively studied 'nodes' of structure, physiology and ecology, speculation is frequent and enticing—the discussions of vertical stratification in ecology, of plastid origin, and of early evolution provide examples.

The two books are complementary as well as overlapping: though the second is a balanced unity, the first contains more detailed accounts of some topics. The coverage achieved will be the envy of addicts to other groups of algae, and the remarkable array of biological characteristics presented will surprise many. To both works is the comment (on the first) of T. D. Brock apt—"the blue-green algae finally come into their own".

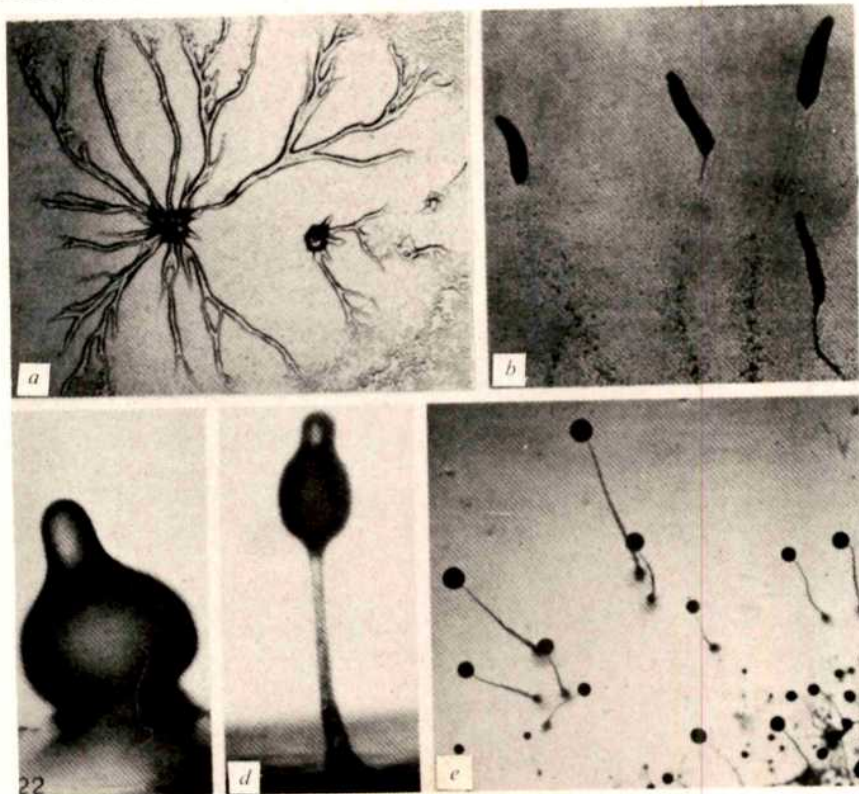
J. F. TALLING

Bigger means better

Evolution of the Brain and Intelligence. By Harry J. Jerison. Pp. xiv+482. (Academic: New York and London, December 1973.) \$25; £11.75.

THIS book covers the evolutionary development of the vertebrate brain. The author's views are expressed very clearly and the whole is well illustrated. A large number of tables and graphs gather to-

Slime mould



STAGES in the differentiation of the cellular slime mould, *Dictyostelium discoideum*, from independent myxamoebae to a multicellular organism containing two cell types. *a*, Aggregating myxamoebae; *b*, migrating 'slugs' containing several thousand cells; *c-e*, differentiation of the slug into two cell types, spore and stalk. From *The Fungi: An Advanced Treatise* (vol. 4B, *A Taxonomic Review with Keys: Basidiomycetes and Lower Fungi*), edited by G. C. Ainsworth, Frederick K. Sparrow and Alfred S. Sussman (Academic; New York and London, December 1973.) \$28.00; £13.15.

gether much of the original evidence and there is a critical handling of the data and its statistical analysis. Since the evidence presented is mainly palaeontological the size of the brain and its outer shape are the main data. From these data are woven a theory of the evolution of intelligence. As the author rightly says "The most controversial facts in the book will probably be associated with the extensive use of the evidence of relative brain size. There is no debate about the validity of the measure. There is a debate, however, about their meaningfulness".

A trivial answer to the meaning of brain size is that it is an index of the number of nerve cells. The evidence for this is presented. It is not of course possible to discuss the degree or nature of interconnection between the nerve cells by using whole brain size. Since this measure cannot be used, the author accepts the conclusion that bigger means better or at least more intelligent.

But before one can proceed to examine which brains are bigger and therefore better, one must first eliminate what might be called the brain/brawn problem because the most obvious factor varying the size of the brain of animals is the size of the animal itself. This

effect is repeatedly and convincingly illustrated. There is a straight-line relationship with a $2/3$ slope between the log of the brain weight and the log of the body weight. This applies within a species and between related species. It even applies over pretty widely scattered species. Fish and reptiles make up one line. Birds and mammals including primates make another parallel line with a bit more brain for each ton of body.

This itself has always seemed to me one of the most surprising facts about brains. Why does a little man have a little brain? Why does it take a huge brain to run a huge dog like a Great Dane whereas a much smaller brain can make a fox terrier or a cairn behave just like a dog. There is no evidence that the Great Dane has more data to analyse in proportion to its body mass except for the relatively small input from his muscles. There is no reason why the motor machinery should need to be expanded as the mass of muscle increases.

In spite of this being the basic fact, it is taken by the author as his base line and he really examines deviations from the base line without really attempting to explain the curious fundamental relationship between brain and body weight.

Fifty million years ago, early horse brains expanded in relation to their body weight but in subsequent horse development the brain increased only in proportion to the body. That early spurt is used to explain why horses are so smart and the later expansion with body size is accepted as a fact of brain life. By implication, the base line represents "the brain tissue needed to transmit impulses to and from the integrative centres" to quote the author's quotation of Lashley. The excess brain is a measure of behavioural capacity in the author's view.

The facts about the growth of brain size are well presented; the meaning of the data is more puzzling. If the excess of brain size beyond that expected for body size were a measure of behavioural capacity, we should be able to spot our academic and political leaders at a glance and they would in fact be the giants they consider themselves to be.

P. D. WALL

Peat

Peatlands. By P. D. Moore and D. J. Bellamy. Pp. vi + 221. (Elek: London, January 1974.) £4.

THE growing interest in peat and in peat-forming ecosystems, both from the ecological and from the commercial points of view, has led to a series of major international peat congresses in recent years. This book, by two of Britain's most knowledgeable and widely-travelled 'peatniks', summarises much of the work that has resulted from these congresses, and provides an invaluable source book on peatland ecology, utilisation, and conservation. In the opening chapters a detailed, and largely original, account is given of the morphologically distinct peatland types recognisable in Europe and North America, and of the climatic, hydrological, and geochemical factors governing their distribution. Of particular interest is the section dealing with the striking surface patterns of peat ridges and open water pools characteristic of some of these peatlands, and with the possible mechanisms by which this patterning arises.

Chapter 4 deals with the processes governing rates of peat accumulation, and with the retention and cycling of mineral ions. Successive chapters then consider structural and physiological adaptations in mire organisms, and the information about peatland development that can be gained from a study of plant remains preserved in the peat. The final chapters discuss the world distribution and exploitation of peatlands, and present a scientific case for more concerted and intensive conservation of peat-forming ecosystems.

Although this book contains much

that is excellent, there is also much that is sub-standard. Surely it was not necessary for the publishers to reduce some of the diagrams to such a size that details are no longer decipherable, nor to split Figs 2.4 and 6.6 each into two halves on non-opposing pages. Misspellings and inconsistencies of usage are frequent, and an almost total lack of hyphens in the text leads to many perplexities: is not the concept of "peat forming vegetation" botanically and grammatically irreconcilable?

This is, then, a book and reference source to recommend to all those interested in peat and peatlands; but one that, because of the abundance of apparently unnecessary ecological jargon in some chapters, will not perhaps be readily comprehended by persons without specialist background knowledge.

JOHN H. TALLIS

Monument to microbes

Microbiology, including Immunology and Molecular Genetics. By Bernard D. Davis, Renato Dulbecco, Herman N. Eisen, Harold S. Ginsberg, W. Barry Wood, jun. and Maclyn McCarty, (Second Edition). Pp. xv+1562. (Harper and Row: Hagerstown and London, 1973.) \$27.50.

THIS is an impressive book in scope, size and authorship. Clearly Gray's *Anatomy* can no longer claim a monopoly that, by bulk and weight, it can simultaneously generate a hernia and profound depression in the medical student who handles it! Any student depression initially engendered by the obvious mass of knowledge purveyed in this volume must surely be offset by the subsequent discovery of a pleasant, readable style, clear, helpful diagrams and illustrations, and a splendid layout.

The vital statistics of this second edition command respect—94 of the 1,562 pages are needed to index its 64 chapters! Substantial revision has occurred and new chapters have been introduced to keep pace with the relentless march of knowledge. The untimely death of Barry Wood necessitated contributions on the pathogenic bacteria from a panel of experts (who reveal their presence by eschewing SI units) under the special editorship of Maclyn McCarty. The book is divided into five principal sections comprising bacterial physiology, bacterial and molecular genetics, immunology, bacterial and mycotic infections, and virology, and, as might be expected from such distinguished authors, the overall treatment is very good, with emphasis on scientific method. The avowed intention to produce a text that tells the medical student not only what is known but how it came to be known, has been

amply realised.

Inevitably in a book of this size there are points to criticise. Thus the claim that bacterial glycogen synthesis involves uridine diphosphate glucose, confusion between free and standard free energy changes, and omission of the unifying concept of adenylate energy charge when discussing metabolic regulation, are some of the blemishes I noticed. But they do not detract significantly from a monumental work which should provide not only students of medicine and the paramedical sciences but also their biological colleagues with a very useful and comprehensive text.

E. A. DAWES

Deep sea fish

Fishes of the Western North Atlantic, Part 6. (Memoir of the Sear Foundation for Marine Research, No. 1.) Pp. xix+698. (Sears Foundation for Marine Research, Yale University: New Haven, 1973.) \$27.50.

THE series of publications under the general title of *Fishes of the Western North Atlantic* is too well known to need introduction. The first volume, produced in 1948, was written entirely by the late H. B. Bigelow and W. C. Schroeder and quickly became a classic source of information on the sharks with which it dealt. It set a standard of excellence which seemed to be almost too high to be maintained in succeeding volumes. Twenty-five years later the sixth part of the series has been published and shows convincingly that the standard is in no way diminished.

Two important policy changes are announced with this volume. First, that in future material will be published as it becomes available rather than in systematic order (and thus waiting for laggard contributors). The second change is to permit publication of new taxa within the volume rather than insisting on prior publication elsewhere. Both changes make good sense as attempts to speed the production of further volumes towards the eventual coverage of the western North Atlantic fish fauna.

Part 6 of the FWNA contains the following contributions: the order Heteromi (Notacanthiformes) by S. McDowell, the suborder Cyprinodontoides (the brackish and salt-water killifishes) by Donn E. Rosen, the order Berycomorphi (Beryciformes) by Loren P. Woods and Pearl M. Sonoda, the order Xenoberyces (Stephanoberyciformes) by A. W. Ebeling and W. H. Weed, and the Macrouridae (rat-tails or grenadiers) by N. B. Marshall and Tomio Iwamoto, preceded by a general account of the order Anacanthini (the Gadiformes or the cod fishes). To

produce a collection of contributions by so many authors is a major feat on the part of the editor Daniel M. Cohen and his editorial board.

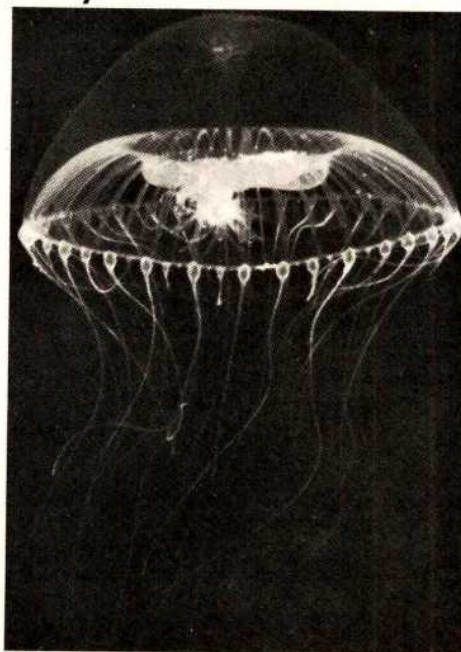
Most of the groups treated in this volume are deep-water fishes which have presented many taxonomic problems in the past. Many of them are also known from relatively few specimens. The value of the volume is therefore very considerable.

McDowell's contribution in unravelling the complicated synonymy of species of spiny eels and halosaurs is a major feat of taxonomy, to which is added a penetrating account of the anatomy and relationships of these fishes. McDowell has summarised the literature and much unpublished information on the distribution and biology, as well as giving detailed accounts of structure and morphology of each species.

Rosen's discussion of the killifishes or euryhaline toothcarps is necessarily different from the other contributions, for this group is primarily freshwater with relatively few euryhaline species. All the marine or brackish toothcarps found on the American Atlantic coast are listed, with notes on their range, clear illustrations, and with a key for their identification.

The berycoid fishes comprise a mixture of deepwater "soldiers", *Hoplostethus* and other genera, together with

Jellyfish



Aequorea victoria, the largest common jellyfish in the area covered by *Seashore Life of Puget Sound, the Strait of Georgia, and the San Juan Archipelago*, by Eugene N. Kozloff. (University of Washington: Seattle, November 1973.) Boards \$15.00; paper \$6.95.

the squirrel fishes (Holocentridae), mainly shallow water tropical fishes. Again, many taxonomic problems were evident in this group which Woods and Sonoda have clarified. It is comforting to see such a troublesome spectre as *Hoplostethus islandicus* of German fishery workers finally laid as a junior synonym of *H. atlanticus*!

The contributions of Marshall and Iwamoto have brought together the widespread literature on the rat-tails (Macrouridae) in a way which will facilitate the identification of these difficult fishes. An elucidation of this group is particularly timely as the rat-tails, the dominant fish group of the lower continental shelf, are becoming increasingly attractive as commercial fishes. Russian exploitation of the northern *Coryphaenoides rupestris* has already reached considerable proportions and wider interest in this relatively well-flavoured fish can be predicted.

ALWYN WHEELER

Taxonomy without genes

Numerical Taxonomy. The Principles and Practice of Numerical Classification. By Peter H. A. Sneath and Robert R. Sokal. Pp. xv+573. (A series of Books in Biology.) (Freeman: San Francisco, October 1973.) £9.40.

THIS is not a second edition of Sokal and Sneath's well known 1963 book on numerical taxonomy but a completely new work. The authors' aim is to provide a comprehensive and up to date account of the subject and to illustrate the advantages of numerical over conventional taxonomy.

It is certainly a very careful and detailed review, and will be of great value to anyone with a professional interest in this field. It is not a book for someone wishing to 'find out about numerical taxonomy'. Readers are referred to the original papers for many of the details and the mathematical notation, although logical and consistent, produces such mind-bending conjunctions as

$$t_{j,1} = \sum_{i=1}^J X_{ij}.$$

When these ingredients are added to the already gross terminology of conventional taxonomy the result is hard going even for people experienced in the field.

The first three chapters on taxonomic principles and evidence provide the background for the rest of the book. The choice and coding of characters is discussed and the authors make it clear that this is the least satisfactory aspect of numerical taxonomy. Chapters 4 and 5 on taxonomic resemblance and structure form the core of the book and chapter 6 deals with methods of esti-

mating cladistic relationships. This last should be particularly useful as it collects together, for the first time, a lot of recently published material. There is a chapter on identification, including the automatic generation of keys; a chapter on populations; a chapter on nomenclature; and a few bits and pieces. The bibliography is vast and there is also a list of applications of numerical taxonomy to systematics, classified by the group of organisms. An author and subject index ends the book.

The authors' basic position (highly paraphrased) is that a purely phenetic classification system would have great advantages for biology in general. Genetic and phylogenetic information should be irrelevant to the classification (however interesting in its own right). Old ideas about phenetic resemblance estimating genetic resemblance have been thrown overboard and the good ship SS Phenetics is now sailing under its true colours. The authors accept that the lack of a satisfactory definition of 'character' is a theoretical weakness, but claim a certain robustness in practice. They also state that, in time, numerical taxonomy will become objective, and criteria for grouping will be agreed by common consent.

The very size and complexity of the book makes this last claim seem unlikely. A tremendous variety of techniques is mentioned and each new alternative to some act which was previously without alternative doubles the number. It may be that a book such as this is an essential first step—an inventory before taking the vote; but it seems to me more likely that what will emerge from numerical taxonomy is a set of very useful mathematical methods but no general agreement about the way in which the results of applying them should be used.

Whether one is sympathetic to the aims of numerical taxonomy or not it must be granted that to have written a book as up to date and comprehensive as this is a remarkable achievement. It will be an indispensable reference work for many years to come, proving useful to all taxonomists, not just those who obey the fiat of pheneticism.

M. HILLS

Mineral metabolism

Calcium and Phosphorus Metabolism. By J. T. Irving. Pp. viii+246. (Academic (Harcourt Brace Jovanovich): New York and London, September 1973.) £15.

INTEREST in this book is quickly aroused by its table of contents since almost every aspect of the subject is covered in an orderly and well-tabulated progression. The style is readable and there is no difficulty in locating particular topics of interest. The book is reasonably

elementary, its depth being necessarily limited by its size, but so much recent knowledge has accumulated, for example with respect to the chemistry of parathyroid hormone, to cyclic AMP and to vitamin D metabolism, that a simple overview such as this is timely. The author might perhaps have made still more use of the opportunities for critical judgment that a monograph provides but attempts to reconcile conflicting evidence are nevertheless made in a rational way. While the overall approach of the book is physiological, biochemical and clinical aspects are interwoven.

The revision for this edition is a little uneven and a few sections are still composed from largely primitive sources. Occasional statements are misleading such as the bald comment that "in myxoedema the level (of blood calcium) is unchanged". The disadvantage of long publication time is apparent particularly in the expanding field of vitamin D metabolism where the most recent references are over 2 years old. Nevertheless this remains an excellent, concise book for students of the field and will also be of value to specialists when they require to look into other physiological aspects of their subject.

T. C. B. STAMP

Ukrainian hunters

Ice-Age Hunters of the Ukraine: Prehistoric Archaeology and Ecology. By Richard Klein. Pp. xviii+140. (University of Chicago: Chicago and London, 1973.) £2.95 boards; £1.35 paper.

THIS small book serves two purposes. First it is a very welcome up to date summary of Soviet research in the upper Palaeolithic of the Ukraine, and, second, it makes available material not readily accessible to Western readers who do not read Russian fluently.

The author takes the occupation of the Ukraine during the Last Glaciation, roughly from about 45,000 to 10,000 years, which covers the Mousterian as well as the Upper Palaeolithic.

The preface and the early sections suggest that the author has undergraduates in mind and this has had some effect on the whole tone of the book, but this approach in no way detracts from its usefulness. Both the archaeology and the supporting studies, geology, fauna and pollen studies, are dealt with in some detail with the result that the area and period are dealt with as a whole, including discussion of the environments during the three stages of the Last Glaciation. This evidence is illustrated by maps, pollen diagrams and faunal lists.

The archaeological side, which is the main purpose of the book, deals very fully with the evidence for dwelling

structures, the earliest of which date from the Mousterian. These houses are mostly constructed from mammoth bone, an animal which forms the bulk of the economy, and whose bones (skulls, long-bones, scapulae and tusks) in a region with a timber shortage, made good building material. In addition to hut plans and their reconstruction, the author reproduces some of the archaeological material, but unfortunately, though being quite generous with his illustrations of the bone objects, gives no useful illustrations of the flint assemblages.

When one looks at the list of references at the back one can only be very grateful for the mass of material that the author has condensed. This is a very useful book and most prehistorians, not excluding Russians, will be glad of it.

J. D'A. WAECHTER

Statistics for biologists

Biostatistical Analysis. By Jerrold H. Zar. Pp. xiv+620. (Prentice-Hall Biological Sciences Series.) (Prentice-Hall: Englewood Cliffs, NJ, 1974.) \$25.95.

WHETHER or not statisticians regard the practice as desirable, many biologists choose to be responsible for their own statistical analyses. To aid them, they have a great many bad and a few good cookery books. Professor Zar's thorough presentation of detailed formulae and abundance of worked examples places him firmly in the second category. Those who are prepared to handle a rather heavily formalised symbolism should welcome this well-produced text. The descriptions of analysis of variance calculations and their associated tests of significance are particularly clear. Perhaps the chief weaknesses are the lack of critical guidance on choice between methods (when should a non-parametric technique be adopted for analysis of a continuous variate?) and the unfortunate habit of presenting the scaffolding of tests of significance without summary tables of means.

More than 200 pages of tables of statistical functions are included. Today's computer facilities, and the availability of keys for standard functions on relatively cheap desk calculators, could have allowed substantial omissions and condensations here, with consequent reduction in the high cost of the book. The attitude to computing, indeed, is surprisingly old-fashioned. Little is said about any sophisticated programming. Such uses of computers as are described are concerned merely with performing the heavy arithmetic and with the barbarity of floating point output.

The book evidently originated in a concern for statistical analysis of observational data rather than planned experiments. This emphasis is valuable,

though it could usefully have been supported by more discussion of sampling of biological populations and materials, selection of observations and measurement procedures. Particularly interesting is the chapter on distributions of angular directions, a topic rarely mentioned in elementary texts.

D. J. FINNEY

Wobble in the glance

Eye-Movements and Visual Perception. by R. W. Ditchburn. Pp. xv+421. (Clarendon: Oxford; Oxford University: London, December 1973.) £9.50.

WHEN we wish to scrutinise an object and look at it in great detail we point our eyes towards the thing and hold them quite stationary—or so one might think. But even during periods of concentrated fixation, the eyes are never really still. They move constantly with three components of motion: a slight tremor (about 50 Hz, amplitude about 20 arc s) probably originating in the intrinsic noise of the extraocular muscles; slow drift (velocity up to 20 arc min s⁻¹); and intermittent, tiny, fast flicks (usually one or two per second, mean amplitude 5 arc min).

Our normal persistent perception of the visual world must depend on one or more of these apparently involuntary movements because if an optical device is used to hold an image more or less fixed on the retina, despite eye movements, the pattern seems to fade virtually completely within a few seconds. Isaac Newton was perhaps the first to recognise this necessity for a moving retinal stimulus. He noticed that pressing the side of the eyeball with a finger stimulates the retina mechanically, producing a coloured blob superimposed on the visual field; however "if the eye and the finger remain quiet these colours vanish in a second minute of time, but if the finger be moved with a quavering motion they appear again"¹.

Since Newton's time there has been more than a little research on the small eye movements of fixation and the curious phenomena that occur when the retinal image is stabilised. Ditchburn's book is a critical catalogue of this work, invaluable to the researcher in this area and of interest to every student of vision. This book, then, has a place as a companion (but not a complete successor) to the highly personal account of eye movements and visual perception by A. L. Yarbus².

Ditchburn begins with a general introduction to visual optics and the problems introduced by movements of the eye. He then provides a most useful review of all the methods of recording eye movements and evaluates them critically.

This section, together with an appendix containing many technical details, is of great value to anyone intending to work in this field where instrumentation is so varied in scope and quality and where the recording apparatus itself imposes severe restrictions on the kinds of movements that can be accurately monitored.

Next comes a survey of the results of these recording experiments with a wealth of quantitative data from many different workers. Ditchburn then discusses the various kinds of apparatus used for producing a more or less stationary retinal image despite movements of the eye. Most involve the wearing of a closely fitting contact lens with various appendages such as a sucker, to hold the lens tightly to the cornea, a mirror on a long stalk or even a miniature projection system complete with lens and light bulb. Some of these devices would have made the Spanish Inquisition green with envy. Unfortunately not all these methods are equally efficient in holding the retinal image stationary: consequently the various introspective reports on changes in colour, brightness and clarity of stabilised images are not all equally valid and it is sometimes difficult to know how to evaluate them.

Before considering the causes of perceptual fading of a stabilised image and the function and control of small eye movements, Ditchburn reviews briefly the relevant physiology of the visual system. But let me be clear: this is not the place to learn about the latest advances in visual physiology. This is perhaps the weakest section of the book, but is adequate for the question in hand.

Ditchburn concludes that no single type of small eye movement (tremor, drift or saccade) is adequate to maintain the normal visibility of the image. He believes that both drifts and saccades are essential. It is worth mentioning that not everyone agrees. Steinman and his colleagues³, for instance, have found that some observers can learn to suppress small saccades completely, without decreasing the accuracy of fixation or the visibility of the target.

Finally, a word about the index. The book is organised into numbered subsections of each chapter and these are used (rather than page numbers) in the index. No doubt this is a great economy in editing and layout but it is a very clumsy system to use. I hope that any future edition of this book may have a real index as well as answering many of the unsolved questions in this fascinating field.

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Fragmentation of the Alpine orogenic belt by microplate dispersal

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Reconstruction of the positions of western Mediterranean microplates in the Oligocene leads to recognition of a formerly continuous 'Alpine' (Mesozoic-Eocene) orogenic belt. This Alpine belt was fragmented by Miocene to Quaternary microplate dispersal in which four episodes of movement are recognised.

It is becoming increasingly clear that the movement of microplates such as Corsica-Sardinia^{1,2} and Spain³ has played a principal role in the tectonic evolution of the Mediterranean but it is difficult to find clear evidence for the exact time and manner in which these and other microplates have moved^{4,5}. Here we attempt to reconstruct the history of late Tertiary microplate movement in part of the western Mediterranean; the evidence will be reviewed in more detail in a forthcoming paper. The first step in this study is to determine the palaeogeography in the Oligocene, before the latest episode of microplate dispersal; the second step is to determine the pattern of movement of the microplates. If the reconstruction given here is correct, it explains the longstanding question of why the Eocene orogenic belt of the Alps terminates in northeast Corsica and clarifies the origin of the Miocene orogenic belt of Italy, Sicily, and North Africa (the Apennine-Maghrebian fold belt).

Oligocene palaeogeography

Figure 1 shows a reconstruction of western Mediterranean palaeogeography in the Oligocene, that is, after the 'Alpine' orogeny in the Cretaceous to Eocene but before the 'Apennine-Maghrebian' deformations of the Miocene. The first step in making this reconstruction is to place Corsica and Sardinia next to Provence. Evidence for this fit has been reviewed¹; further evidence from palaeomagnetism^{6,7} and geological matching⁸ has been reported. Corsica and Sardinia are separated by the Straits of Bonifacio, an opening which widens westwards and which may be a 'microspinochasm'^{4,8}. A greater rotation for Sardinia than for Corsica has been assumed here⁸ in order to close the Straits of Bonifacio, improve the geometrical fit with France and better satisfy the palaeomagnetic data⁷. The 'eugeosynclinal' sequences occurring as allochthonous nappes and slabs in the northern Apennines were originally deposited in the area between Corsica and Italy^{9,10}, and the internal (that is, originally south-western) parts of the eugeosyncline must have been within the Alpine belt since they were deformed

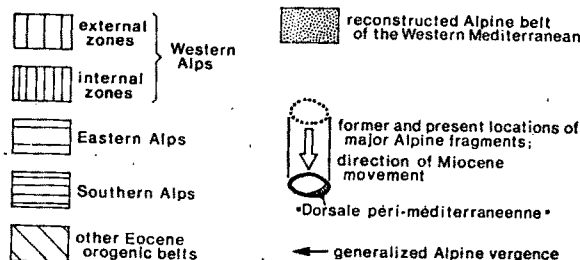
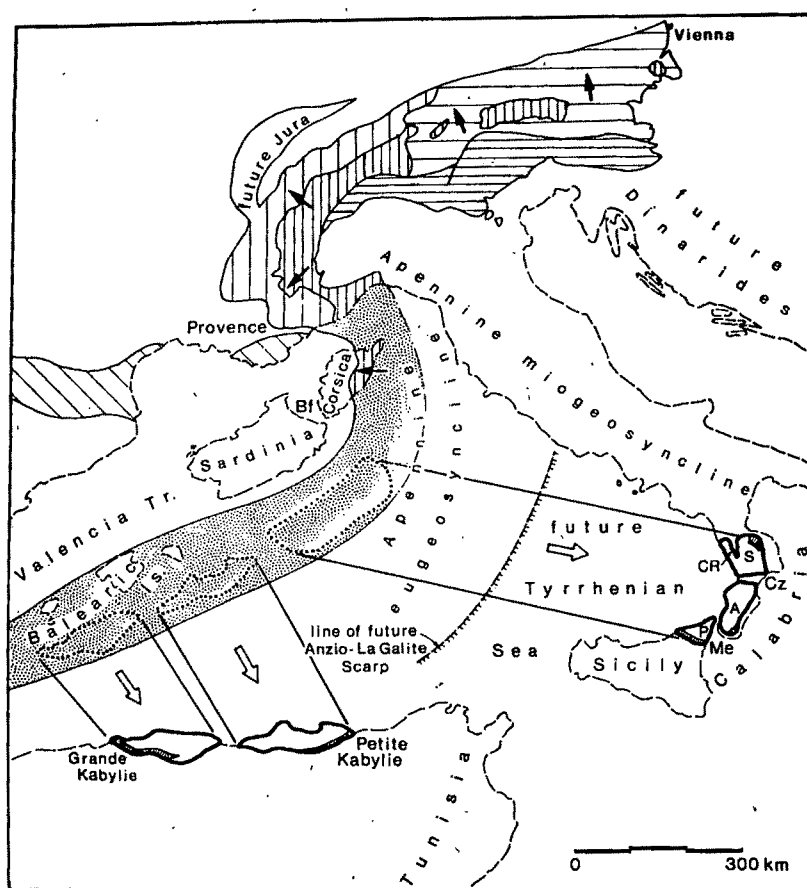
in the Palaeogene¹⁰⁻¹². The external parts of the eugeosyncline lack a Palaeogene unconformity and were thus outside the Alpine belt.

As the next step we propose that the crystalline Calabrian 'massif' of southernmost Italy and NE Sicily should be placed next to Sardinia. This massif, 200 km long and 50 km wide, links the NW-SE trending Apennines with the east-west Maghrebian trends. It is a unique element in the otherwise sedimentary sequences of the Apennines, but most of its characteristics are repeated in the two Kabylie massifs of Algeria and the Rif massif of Morocco. The Calabrian massif is split into three segments by the Straits of Messina and the Catanzaro lowland; we suggest that these transverse depressions may be 'microspinochasms' due to fragmentation of the massif during its south-eastward movement.

Evidence for the former position of Calabria shown in Fig. 1 is provided by (1) similarities with Alpine Corsica; (2) sediment supply requirements; (3) similarities with the Kabylie massifs; (4) the present movement of the massif and (5) displacement of the Numidian flysch-crystalline massif suture 300 km south-eastwards from Tunisia to NE Sicily (discussed in the final section).

(1) Calabria is not, as has often been implied, a Hercynian massif unaffected by tectonism since the end of the Palaeozoic. Rather it is a stack of nappes (some containing Hercynian crystalline rocks) that, according to some authors¹³⁻¹⁵, were emplaced during the Eocene, that is, at the same time as the nappe emplacement in NE Corsica¹⁶. The direction of movement of the Calabrian nappes is currently a matter of debate, so this aspect cannot be compared with Corsica, but there are close analogies in lithology and metamorphism (high *P/T*) between Calabria and NE Corsica. Both areas contain phyllites and schists with ophiolites and metabasic rocks—the 'schistes lustrés' of Corsica¹⁷⁻²⁰ and various units with controversial nomenclature, some of which have been referred to as 'schistes pseudolustrés'²¹, in Calabria^{13,22-24}. Blueschist metamorphism is found in both areas^{13,24-26}; elsewhere in the western Mediterranean it is known only in the French Alps²⁷ and in the Betic Cordilleras²⁸. All four areas are part of the reconstructed Alpine belt of Fig. 1.

(2) Two sedimentary units on or adjacent to the Calabrian massif (Fig. 2) seem to require sources of detrital material in Sardinia: the Tusa andesitic flysch, recently shown to be late Oligocene-Aquitainian in age²⁷ seems to be a trench deposit^{30,31} and the late Oligocene-early Miocene volcanic province of western Sardinia seems to be the only available source of calc-alkaline detritus^{29,30}; the transgressive Capo d'Orlando flysch, which covers the NE Sicilian part of the Calabrian massif and is probably an arc-trench gap deposit²⁹,



contains clasts of a red porphyry found in place in Sardinia but not in southern Italy or Sicily³².

(3) We also note the similarity between the Calabrian and the Kabylie massifs in orogenic timing^{33,34} and in the precisely analogous Mesozoic cover of the 'Dorsale péri-méditerranéenne'³⁵ ('Chaîne calcaire') in the two areas^{33,36}. The 600 km gap between these massifs and the northward projection of the Tunisian shelf make either submerged continuity or separation as a result of east-west motion highly unlikely, but the similarities are explained well by the diverging motions shown in Fig. 1.

(4) The active volcanic and seismic zone beneath Calabria reveals ESE-directed motion of the massif³⁷⁻⁴⁰, indicating that Calabria is at present moving in the direction required by the reconstruction of Fig. 1.

A recent report of basement nappe movement of Alpine age in eastern Sardinia⁴¹ suggested that Alpine Calabria might have been located very close to Sardinia. Our field results⁴² however, show that the 'basement nappe' is simply a massive landslide of incompetent phyllite off the side of an early Tertiary horst. Thus Sardinia must have been well outside the zone of Alpine compression. We tentatively assume that the elongated seamounts of the Monti di Quirra and the Monti delle Baronie, which trend north-south about 50 km east of Sardinia⁴³ and from which crystalline rocks have been dredged⁴⁴, are part of the Sardinian continental crust. This suggests that the Calabrian block should be placed east of these seamounts, 50-100 km east of the present coast.

FIG. 1 Reconstruction of the locations of western Mediterranean microplates during the Oligocene after the final Alpine phase in the Eocene but before the Apennine-Maurebian movements in the Miocene. The probable closure of the Valencia trough has not been shown since data for accurate reconstruction are lacking. This interpretation uses a greater angle of rotation for Sardinia than for Corsica, thus closing the probable sphenochasm of the Straits of Messina (Bf). The present positions of the Alpine fragments are shown by heavy lines. The Calabrian 'massif' is broken in three parts by the Catanzaro (Cz) and Messina (Me) sphenochasms. CR, Coastal Range; S, Sila; A, Aspromonte; Serra San Bruno; P, Peloritani Mountains.

Although less evidence is available than for the preceding fit, we make an analogous reconstruction to the southwest, first closing the Valencia trough⁴⁵ and then placing the Kabylie massifs against the Balearic Islands on the basis of the Calabria-Kabylie similarities already noted and of the Sardinia-Kabylie similarities discussed by Vardabasso⁴⁶.

During the Oligocene and early Miocene there must have been an oceanic basin south-east of Calabria with the Tuscan andesitic flysch⁴⁷, discussed previously, deposited in a trench on the north-west side, and the continental rise deposits of the Numidian flysch on the south-east side³⁰. According to one of us, the Numidian quartz sands were derived from the Saharan shield⁴⁸⁻⁵⁰. (Arguments, based on thermoluminescence, for a Sardinian source for the Numidian quartz have recently been shown to be invalid⁵¹.) The distribution of the Numidian shows that the Oligocene African continental margin extended from southern Spain and Morocco through north Africa and Sicily to the Anzio-Ancona line in central Italy^{20,49}. Not all this margin however, was necessarily backed by African sialic crust; it has been proposed^{4,52} that in the Jurassic an ocean basin opened between Sicily and Italy, so oceanic crust would have been continuous from the site of the present Tyrrhenian Sea into the eastern Mediterranean.

Characteristics of reconstructed Alpine belt

In plate tectonic terms the Alpine orogenic belt is generally considered to be a suture marking the closure of a small

ocean that opened in the Jurassic and early Cretaceous and closed with major episodes of crustal consumption in the middle of the Cretaceous and the Eocene⁵². The Alpine belt as reconstructed in Fig. 1 comprises the Alps proper and NE Corsica, then trends SW through Calabria and the Kabylies, and may continue into the Betic Cordilleras of southern Spain, although the orogenic history of the latter area is not yet well understood^{53,54}. From the western Alps as far as the northern part of the Calabrian massif (Coastal Range, west of the Sila) the Alpine belt is characterised by 'schistes lustrés', ophiolites, and blueschist metamorphism. From the northern Sila (Longobucco⁵⁵) through NE Sicily and the Kabylies to the Rif and the western Betic as far as Granada⁵⁵, a new element, the 'Dorsale péri-méditerranéenne' or 'Chaîne calcaire', is continuously present^{56,58}. If this change had occurred between two Alpine fragments, it might have cast doubt on their continuity. But, since western Alps-type characteristics and the 'Dorsale péri-méditerranéenne' overlap within a single Alpine fragment and since Eocene orogeny is characteristic of all the fragments, the change does not argue against the proposed reconstruction of the Alpine belt.

The reconstructed Alpine belt of Fig. 1 trends WSW from Vienna to the Kabylies and possibly to Gibraltar, with an offset in the French Alps. Study of magnetic anomalies in the Atlantic⁵⁷ has permitted Pitman⁵² to determine the successive poles of Africa-Europe rotation. From the early Eocene (Ypresian; anomaly 21, 53 m.y.) to the Tortonian (anomaly 5, 9 m.y.) the average pole was at about 34.4°N, 2.5°W in the European reference frame, or about 300 km southeast of Gibraltar, and Europe and Africa approached each other with a rotation of 6.8°. This rotation probably took place mostly in the Eocene, a time of intense tectonic activity. An Africa-Europe rotation of 6.8° about this pole would cause crustal shortening of 100–200 km in the Alps, which may be compared with geological estimates ranging from 100 to 600 km⁵⁸. Thus the Alpine belt was apparently formed by crustal shortening and/or consumption during the approach of Europe and Africa; this is, of course, the classical explanation of the Alps proper.

The offset in the Western Alps may be due to a projection of the front of the overriding African plate, that is, the pro-

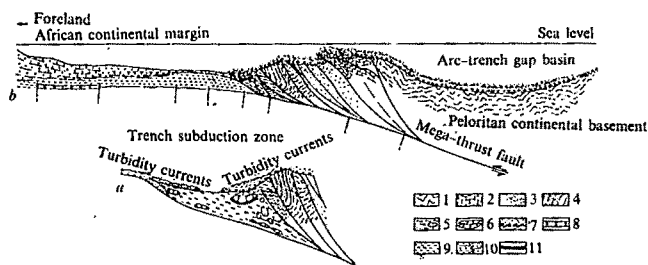


Fig. 2 Front of the rotating Sardinia-Calabria microplate during the early Burdigalian (second episode of movement). This reconstruction is based on geological relationships in NE Sicily and shows the Peloritan basement (Sicilian part of the Calabrian 'massif') overriding oceanic sediments north of the African continental margin prior to collision with Africa in the early Langhian. *a*, Detail of the trench shown in *b*. 1, crystalline basement; 2, limestones of the 'Dorsale péri-méditerranéenne'; 3–6, flysch and mélanges on the south side of the Calabrian massif; 7, Reitano-Capo d'Orlando flysch (arc-trench gap turbidites); 8, Subsided Panormide carbonate platform; 9, Pre-flysch of the African continental margin; 10, Numidian continental-rise sediments; 11, Radiolarites and diatomites related to volcanic episodes. This cross section is discussed in detail by Wezel²⁹.

jecting Italian block may have pushed the main part of the Alps further to the north. Alternatively, the offset may reflect a trench-trench transform fault following a small circle having its centre at the pole of rotation near Gibraltar. A

third possibility is that the Corsica-Sardinia block was part of the Iberian plate while the latter was rotating during the Eocene³ and that as this plate moved eastward it distorted the Alpine belt and produced the curvature.

Fragmentation and dispersal of microplates

In France, Switzerland and Austria the Alpine suture was caught between the two large sialic blocks of Europe and Italy. In the western Mediterranean it seems that an oceanic area remained not far southeast of the suture^{4,52} and that the microplates moved into this area. The reason for the microplate movement is not clear, although it may be related to buoyancy of crustal material subducted earlier, during the suturing. It has been suggested^{59–61} that the movements are due to back-arc extension or 'multisubduction' similar to that of the western Pacific⁶². We note, however, a possible difference: while in the western Pacific general subduction of the Pacific plate under Asia produces local upwellings that drive the island arcs back over the Pacific plate, in the Mediterranean there does not seem to have been a general subduction (for example, at present, Calabria alone is moving ESE over the Ionian Sea^{37,40}). It seems that the upwelling is the primary driving mechanism, producing only local subduction. Thus in a given episode we expect to find first volcanism, then crustal separation and microplate movement with local subduction.

On the basis of available data four episodes of microplate movement can be tentatively identified in the Tyrrhenian Sea area. The first two episodes probably had analogues to the southwest in the Balearic-Kabylie region; the third and fourth episodes are much more difficult to interpret than the first two.

The first episode involved initial rotation of the Corsica-Sardinia-Calabria microplate away from France and formation of the first part of the Ligurian Sea behind the rotating microplate. The beginning of this episode is marked by the initiation of andesitic volcanism in Sardinia in the late Oligocene—a volcanism which continued until the beginning of the middle Miocene⁶. Graben formation accompanied the volcanism in the Oligocene^{63,64} but crustal separation in the Ligurian Sea area need not have occurred immediately. We propose that the separation came in the Aquitanian, since this is the time of a rapid, regional, marine transgression over continental deposits in the Campidano lowland of SW Sardinia, an early Tertiary graben which was ideally situated to record a marine incursion at the beginning of rotation^{65,66}. (Analogous opening of the Valencia Trough probably occurred at the same time; this is supported by an age date of 22–19 m.y. on andesitic rocks cored at DSDP site 123 (ref. 45).) The end of the first episode came during the late Aquitanian when Corsica collided with the sialic crust of the northern Apennines, a collision marked by orogenic activity in the eugeosynclinal sequences of the northern Apennines^{12,67}.

Corsica was then unable to rotate further and the second episode began as the Sardinia-Calabria microplate broke away from Corsica and continued to rotate, forming the rest of the Ligurian Sea and the microsphenochasm of the Straits of Bonifacio. This separation took place in the early Burdigalian, as is shown by marine transgressions of that age in the Bonifacio basin of southern Corsica⁶⁸ and in its continuation, the Capo Testa basin of northern Sardinia⁶⁹. Volcanism related to this opening cannot be separated from the rest of the Sardinian Oligo-Miocene volcanism. The palaeomagnetism of volcanic rocks intercalated with fossiliferous marine sediments at Castelsardo in northern Sardinia indicates that Sardinia rotated rather suddenly in the early Miocene⁶; further studies⁷ suggest rotation in the late Aquitanian. These dates agree well with the timing proposed here. Figure 2 shows conditions at the leading edge of the Sardinia-Calabria microplate during the second episode of rotation, with the Peloritan continental basement (part of the Calabrian

'massif') approaching the African continental margin. This second episode terminated with the collision between the rotating microplate and the Tunisian margin of North Africa. In this collision the NE Sicilian part of the Calabrian massif (Peloritani Mountains) overrode the Numidian flysch deposits of the African continental slope—orogenic activity that

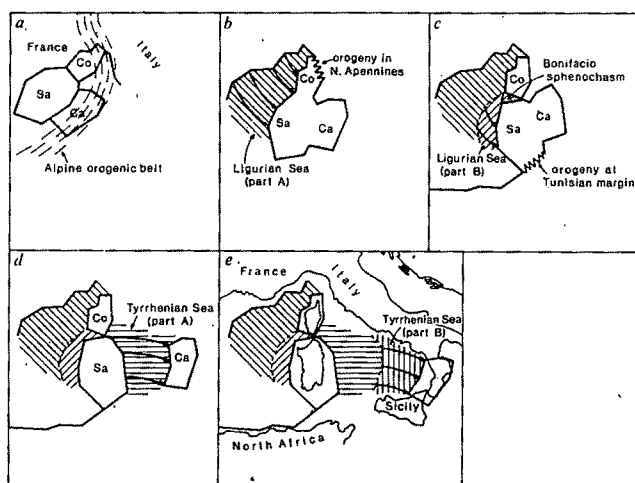


FIG. 3 Schematic diagrams to show the four episodes of tectonic evolution proposed in this paper. *a*, Reconstruction end Oligocene. *b*, After first episode, late Aquitanian. *c*, After second episode, early Langhian. *d*, After third episode, Tortonian. *e*, After fourth episode, present day. Co, Corsica; Sa, Sardinia; Ca, Calabria.

can be dated²⁹ as early Langhian (upper part of zone N-8 of Blow⁷⁰, about 14 m.y.). Collision of the independent but analogous Kabylie microplate(s) with North Africa took place very slightly earlier (lower part of zone N-8 of Blow, about 14.5 m.y., M. Durand-Delga, personal communication, 1973). The line of collision of the Sardinia-Calabria microplate with North Africa is preserved today as the submerged boundary between the Sardinian crystalline block and the Numidian flysch in the Straits of Tunisia^{71,72}. Collision of the rotating microplate with the continental mass of North Africa seems to have stopped the rotation of Sardinia, while the Calabrian portion of the microplate, facing the oceanic crust of the eastern Mediterranean-Ionian Sea, broke free and continued to move to the south-east, carrying the line of collision of the Peloritani Mountains with the Numidian about 300 km south-east to its present position.

If the reconstruction of Fig. 1 is correct, the Tyrrhenian Sea was formed in the wake of the Calabrian massif as it moved south-eastwards, but general agreement has not been reached on the time and mode of origin of this sea, and the question of spreading as against oceanisation in place is a matter of continuing debate^{45,73,75}. There are conflicting views on the age of the basin. At DSDP site 132 upper Miocene (Messinian) shallow-water evaporites are abruptly overlain in transgression by deep-water Pliocene pelagic ooze ('Trubi'); this led to the interpretation, generalised for the entire Tyrrhenian Sea, that the basin was formed before the Messinian, was dried out by evaporation during the Messinian and was suddenly flooded at the beginning of the Pliocene⁴⁵. On the other hand, a widespread Middle Pliocene transgression analogous to the Middle Pliocene transgression seen on land⁷⁵, has been suggested on the basis of dredge hauls and seismic reflection profiles⁴⁴, implying that the present basin is Middle Pliocene or younger. We suggest a possible reconciliation of these views by noting that the Tyrrhenian Sea is divided into two parts by a NE-SW trending boundary, here called the 'Anzio-La Galite Scarp'. To the north-west of this scarp is an abyssal plain without volcanoes at about -2,800 m that is underlain by horizon 'M' (top of the Messinian evaporites); DSDP site 132 is in

this area. North-south ridges^{43,74} emerge northwards from this abyssal plain, apparently forming a continuation of the tectonic ridges of the northern Apennines. To the south-east of the scarp is an abyssal plain at about -3,400 m, marked by large Plio-Pleistocene volcanoes with a NNE-SSW elongation^{43,74}. We have not been able to trace the distinctive seismic horizon 'M' across the Anzio-La Galite Scarp into the SE Tyrrhenian, nor to recognise it anywhere in this area. Thus we tentatively conclude that the Messinian evaporites are not present in the SE Tyrrhenian, at least not in the very thick, evaporated, shallow-water deep-basin facies of DSDP site 132. The SE Tyrrhenian basin may thus be post-Messinian. The possible difference in age between the two parts of the Tyrrhenian basin leads us to suggest two further episodes of microplate movement, although these are more speculative than the first two.

After collision with Tunisia the Sardinian part of the microplate was no longer free to move and the third episode began with separation of Calabria from Sardinia. This separation must have been post-Aquitian (if the Sardinian volcanics were in fact the source of the latest Oligocene-Aquitian Tusa andesitic flysch on the south-east side of the Calabrian massif⁴⁷), later than early Langhian (assuming that Sardinia and Calabria were attached until Sardinia was blocked by Tunisia) and pre-Messinian (because Messinian evaporites were deposited in the western Tyrrhenian at DSDP site 132 ref. 45). The Miocene delta of Orsei, on the east coast of Sardinia, has been dated as Helvetian (Middle Miocene), and interpreted as marking the initiation of the north-south horst of eastern Sardinia⁷⁶. Thus we suggest that the separation of Calabria from Sardinia took place in the middle Miocene, and that Tortonian deformation in Sicily⁵⁰ indicates a collision that terminated this phase of separation. The part of the Tyrrhenian Sea west of the Anzio-La Galite scarp would have formed during this third, pre-Messinian, episode of movement and would then have been the site of Messinian evaporite deposition.

The fourth episode occurred from the Pliocene to the Recent, with Calabria moving ESE to its present position. The movement of the Calabrian microplate may be reflected by greater fragmentation of the allochthonous Apennine carbonate block mountains from NW to SE: in the central Apennines the allochthonous masses average 30 × 100 km; in the southern Apennines they are smaller, averaging 10 × 40 km. The big central volcanoes of the SE Tyrrhenian, M. Vavilov and M. Marsili, are probably related to the motion of Calabria. They are located on the WNW-ESE axis of the basin along the track probably followed by Calabria (the up-dip direction of the present seismic zone) and are elongated at right angles to the basin axis^{43,44}. Dredge samples show that the volcanic rocks of M. Marsili are tholeiitic and have affinities with those of mid-ocean ridges⁷⁷. Also noteworthy is the remarkably circular pattern of the south-easternmost Tyrrhenian, with the inner ring of the Aeolian Islands-M. Palinuro and the outer ring of the southern Apennines-Calabria-northern Sicily mountains concentric about the Marsili volcano. These observations suggest that M. Marsili and M. Vavilov may mark centres of accretion in a complex, non-linear, pattern of behind-the-arc seafloor spreading.

Continuing activity is shown by the high heat flow of the Tyrrhenian Sea⁷⁸ and by the active seismic zone dipping WNW beneath Calabria and the associated volcanoes of the Aeolian Islands⁸⁷⁻⁹⁰. This narrow seismic zone must be interpreted as showing ESE-directed overriding of a small Calabrian plate, rather than general subduction of Africa, beneath Europe³⁷.

Relation to magnetic studies

Bayer *et al.*⁷⁹ give a magnetic anomaly map of the western Mediterranean and a tectonic interpretation based on this

map. Their interpretation agrees with ours in placing the Kabylie massifs among the Mediterranean microplates but differs by placing the poles of rotation of Corsica and Sardinia relative to Europe about 60° away so that there is little change in orientation of the islands during their movement. We have used poles nearby so that the islands have large total angular rotations: 32° (Corsica) and 65° (Sardinia)⁴. The palaeomagnetic results showing large rotations of Corsica⁸⁰ and Sardinia⁸¹ are dismissed by Bayer *et al.*, who note that the islands must first have rotated with Iberia; they attribute the 30° palaeomagnetic declination change in Corsica to this Iberian rotation and state that the larger rotation of Sardinia is not compatible with their data. But the Permian declinations from Corsica differ from those of the Esterel Massif in Provence⁸⁰, which must also have rotated with Iberia since the north Pyrenean transform cannot be drawn between Corsica and the Esterel in the pre-Miocene reconstruction without destroying the striking geological match^{45,80}. Thus the palaeomagnetic data indicate that the islands have rotated with respect to southern France.

We consider the interpretation of the magnetic anomalies to be open to some doubt because of the low amplitude and ambiguous pattern of the anomalies and the inherent problem of correlating the indistinct magnetic profile between anomalies 5 and 6 (10–20 m.y.—the probable age of the Corsica-Sardinia movements) across transform faults. In view of the apparent absence of well developed symmetrical anomaly patterns in marginal basins⁸² particular caution should be exercised in interpreting the anomalies of the western Mediterranean, which shows a number of the characteristics of marginal basins. Herron *et al.*⁸³ emphasise that completely erroneous interpretations can result from incautious use of marine magnetic data. Careful re-examination of both the palaeomagnetic results and the marine magnetic data from the western Mediterranean is clearly necessary.

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Size and folding of the messenger for phage T4 lysozyme

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The existence of non-random base pairing in T4-specific mRNA is supported by the nuclease digestion patterns of the messenger for phage T4 lysozyme. Under conditions which denature RNA, the nuclease nicked lysozyme mRNA recovers its activity. This behaviour can be explained by a model in which secondary structure controls initiation at existing ribosomal attachment sites.

We have reported techniques for assaying the biological activity of lysozyme messenger RNA in an *in vitro* system¹, and by use of this technique were able to show in preliminary studies^{2,3} that the lysozyme mRNA activity is found in molecules much larger than the lysozyme structural gene alone.

We now show that lysozyme mRNA activity extracted from infected cells occurs in molecules of several discrete sizes. The lysozyme messenger activity of the larger molecules can be destroyed by treatment with T1 RNase *in vitro*. These inactivating cleavages can occur at specific locations outside the structural gene for lysozyme as shown by the fact that, on heat disaggregation, biological activity (for lysozyme synthesis) is recovered in smaller molecules of discrete size classes. We have previously shown by several independent methods that T4-specific mRNAs contain a large amount of highly ordered base-pairing (submitted for publication.) The experiments described here provide further evidence that T4 mRNA molecules have a well defined secondary structure and we discuss the implications of this evidence for processing of bacteriophage mRNA molecules *in vivo* and for control of translation by mRNA secondary structure.

Translation of lysozyme mRNA fragments

RNA extracted 25 min after infection of *E. coli* B⁺ with phage T4D+ can code for lysozyme synthesis in an *in vitro* system^{1,2}: Fig. 1 shows that digestion with increasing amounts of RNase T1 progressively destroys this ability. To analyse the effects of such digestions on the sizes of the surviving lysozyme mRNA molecules, we have sedimented such preparations on sucrose gradients (Fig. 2). Before nuclease treatment, the RNA was heated at 80° C for 2 min in a low ionic strength buffer containing 0.01 M EDTA, 0.01 M Tris-Cl, pH 7.4 (measured at room temperature), then quick-cooled. The purpose of this procedure, 'heat disaggre-

gation', was to dissociate any aggregates and to reveal any pre-existing 'hidden breaks'. The preparation was then sedimented either without digestion (Fig. 2a), with digestion (Fig. 2b and d), or with digestion followed by a second heat disaggregation (Fig. 2c and e).

In the undigested control sample (Fig. 2a), lysozyme mRNA activity sediments in discrete multiple peaks. Other experiments involving sedimentation in denaturing solvents indicate that active lysozyme mRNA exists in a number of discrete size classes (our unpublished results). Although RNase T1 digestion inactivates the lysozyme mRNA, the activity has a sedimentation profile similar to the control and, in particular, there is no indication that activity is shifted to slower sedimenting peaks (compare Fig. 2b and d with Fig. 2a).

This is the result expected if lysozyme mRNA is tightly folded with a great deal of base pairing. In such a situation, many of the single-strand breaks would be masked so that a lysozyme mRNA molecule containing several nuclease cleavages could be held together by base-pairing, and sediment as a single unit. The results in Fig. 2c and e show that the nuclease-treated molecules do contain hidden breaks: if the nuclease treatment is followed by a second heat-disaggregation these breaks are revealed and the lysozyme mRNA activity is found in smaller fragments sedimenting at about 13S and 18S (compare Fig. 2c with Fig. 2b and Fig. 2e with Fig. 2d). This suggests that the lysozyme mRNA has a well-defined secondary structure in which a small number of very susceptible regions ('hotspots') are preferentially cleaved. If this were not the case, then we would expect the nuclease treatment to yield a broad distribution of fragment sizes with no definite peaks.

Prolonged heating at 80° C destroys lysozyme messenger activity (unpublished data of B. R.). For our heat disaggregation steps, we have used very short heating times to minimise the amount of thermal inactivation. Since Möller and Boedtker⁵ have suggested that heating causes chain breaks, there remained the possibility that thermal chain breaks might explain some of our results. To test this, we have investigated the effect of heating for various lengths of time on the sedimentation pattern. The results show that the main effect of heating is to disaggregate and reveal hidden breaks: the changes in sedimentation pattern which do occur on heating occur very rapidly and are independent of the time of the heat treatment. Thermal breaks, if they occur, should continue to accumulate throughout the heat treatment. After 10 min of heat treatment, which inactivated 74% of the lysozyme messenger activity, there was no indication of thermal breaks in either the sedimentation profile of lysozyme mRNA activity or in the ultraviolet absorption profile of

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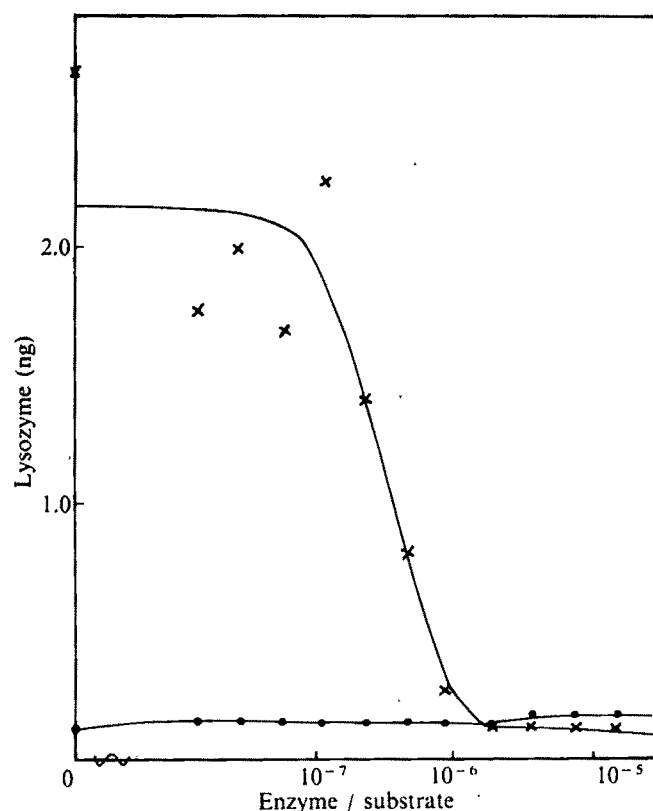


FIG. 1 130 μ g RNA, extracted either 5 min (●) or 25 min (X) after infection of *E. coli* B⁸ with T4D⁺, at a concentration of 4.8 mg ml⁻¹ was treated with RNase T1 in 0.2 M NaCl, 0.02 M magnesium acetate, 0.05 M Tris-Cl, pH 7.5, in the presence of 50 μ g ml⁻¹ bovine serum albumin (BSA). The largest enzyme to substrate concentration was 1/130,000. After a 10 min incubation at 37° C, EDTA was added in stoichiometric amounts to the Mg²⁺ and the RNA tested in a protein synthesising system *in vitro*.

rRNA. Apparently, most of the thermal inactivation of mRNA which we see must be due to some process which is considerably more rapid than thermal chain breakage, although much slower than heat disaggregation.

Heating activates nicked mRNA

Not only does heat disaggregation cause nuclease-treated lysozyme mRNA activity to shift from rapidly sedimenting complexes to smaller fragments, but also some of the lysozyme messenger activity which was originally lost in the nuclease treatment is recovered (Fig. 2). If we take the original lysozyme messenger activity to be 100%, then the preliminary heat disaggregation reduces the activity to 72%. Subsequent nuclease treatment at an enzyme to substrate ratio of $1:3.2 \times 10^6$ (w/w) causes the activity to decrease to 40% of the original. But a second heat disaggregation causes a 1.4-fold increase in the activity, to 56% of the original. With twice as much nuclease the enzyme activity drops to 21%, but the second heat disaggregation causes a 1.55-fold increase in activity, to 32.5% of the original activity.

These increases in enzyme activity are even more impressive when we consider that the normal effect of the heat treatment alone is to cause a roughly 21% inactivation of messenger activity (unpublished results of B.R.). Corrected for this factor the 1.4-fold activation noted above would be a 1.77-fold increase in activity and the 1.55-fold activation would be a 1.96-fold increase in activity. The stimulation of ability to make active lysozyme resulting when nuclease-treated mRNA was disaggregated was confirmed in other experiments involving heat treatments and immediate assay without sedimentation on sucrose gradients. One sort of model which can explain this type of result is shown in diagrammatic form in Fig. 3. In this hypothetical model it is

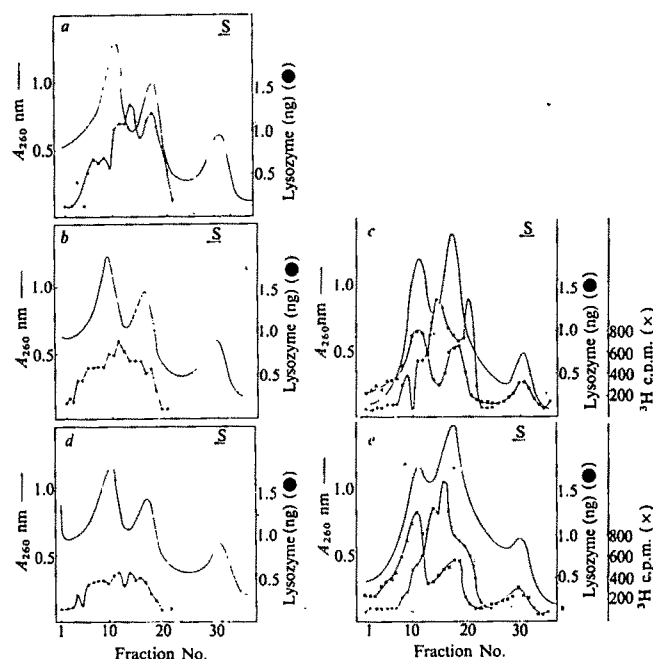


FIG. 2 a, One mg 25 min RNA was heat disaggregated, adjusted to 1 ml in 0.1 M NH₄Cl, 0.01 M ammonium acetate, pH 4.6 and layered on a 36 ml 5–20% sucrose gradient made with RNase-free sucrose in the same buffer. Centrifugation was in a Spinco SW27 rotor at 27,000 r.p.m. for 17 h at 4° C. Fractions of 1 ml were collected and the RNA was precipitated with 2 volumes cold ethanol and 0.1 volumes of 3 M sodium acetate, pH 5.1. The RNA was left at –20° C overnight and the precipitated RNA was pelleted in a clinical centrifuge at top speed. The pellets were allowed to drain for 1–2 h at 4° C and then were dissolved in 50 μ l of sterile, doubly distilled water. A sample of 25 μ l was assayed for lysozyme mRNA activity. The recovery of activity was 72%. Note that in a, as well as in b and d, in which there was only a single heat disaggregation step, there is still some aggregation of rRNA (as shown by ultraviolet-absorbing material sedimenting faster than 23S). We believe that the sedimentation pattern which we observe for the lysozyme mRNA activity is not due to aggregation since the peaks observed after heat disaggregation are similar to those observed during sedimentation in denaturing solvent (DMSO gradients) or when the mRNA has been partially purified before centrifugation so that aggregation with rRNA cannot occur.² In any case, the most important conclusions which we wish to draw are based on changes in the sedimentation profiles of active lysozyme mRNA after the various treatments and these conclusions are largely independent of whether some aggregation is occurring under the sedimentation conditions used. —, $A_{260\text{ nm}}$; ● — ●, ng lysozyme synthesised. b, One mg 25 min RNA which had been heat disaggregated was digested with RNase T1 as in Fig. 1 and layered onto a sucrose gradient without further heat treatment. The enzyme to substrate ratio was $1:3.2 \times 10^6$. After sucrose gradient analysis the recovery of activity was 40% of the original. —, $A_{260\text{ nm}}$; ● — ●, ng lysozyme synthesised. c, One mg 25 min RNA was heat disaggregated and digested with RNase T1 as in (b). Then, to unmask hidden breaks, the RNA was precipitated (by adding 2 volumes ethyl alcohol and 0.1 volumes 3 M sodium acetate, pH 5.1 and centrifuging after 30 min at –20° C), resuspended in 0.5 ml 0.01 M Tris-Cl, pH 7.4, 0.01 M EDTA, and again heat disaggregated. The RNA was then layered onto a sucrose gradient and centrifuged as in (a) with ³H-labelled stable RNA added to provide sedimentation markers. The recovery of activity was 56% of the original. —, $A_{260\text{ nm}}$; ● — ●, ng lysozyme synthesised; X—X, ³H-labelled stable RNA markers. d, One mg 25 min RNA was heat disaggregated, then digested with RNase T1 at an enzyme to substrate ratio of $1:1.6 \times 10^6$. After sucrose gradient analysis, the recovery of activity was 21% of the original. —, $A_{260\text{ nm}}$; ● — ●, ng lysozyme synthesised. e, One mg 25 min RNA was heat disaggregated and digested with RNase T1 as in (d), then reheated in low salt to unmask hidden breaks and centrifuged with ³H-labelled stable RNA markers as described in (c). The recovery of activity was 32.5% of the original. —, $A_{260\text{ nm}}$; ● — ●, ng lysozyme synthesised; X—X, ³H-labelled stable RNA markers.

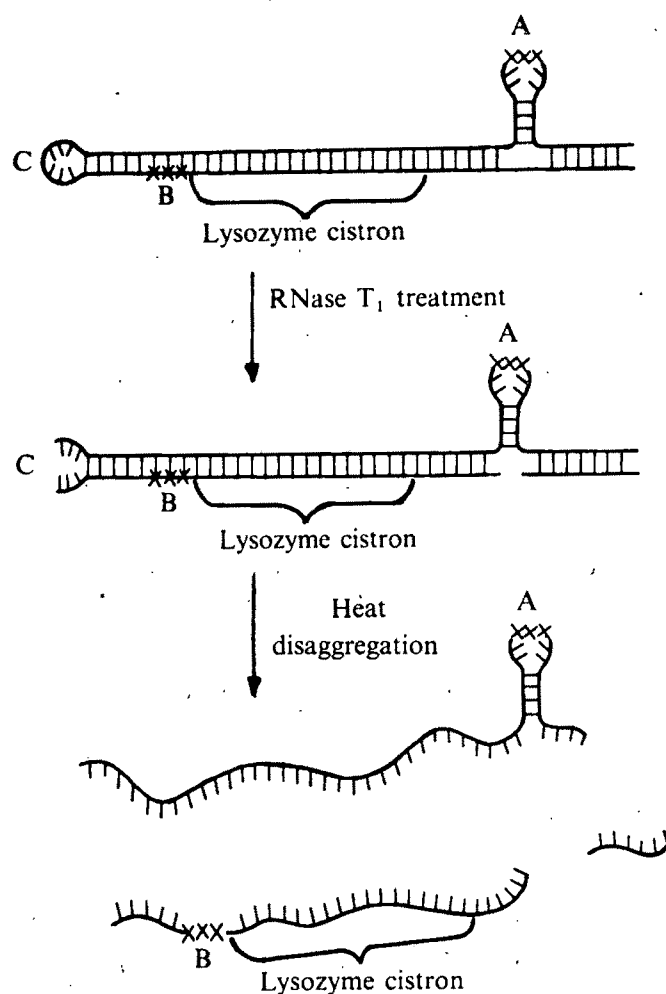


FIG. 3 Model showing how heating could reactivate nuclease-inactivated mRNA by exposing base-paired ribosomal attachment sites.

imagined that the rapidly sedimenting lysozyme messengers contain (at least) two ribosomal attachment sites, A and B. Lysozyme synthesis is carried out by ribosomes which attach at ribosomal attachment site A, which is exposed, and continue on into the lysozyme cistron. Ribosomes cannot attach at site B because it is in a base-paired configuration. RNase, by cleaving the loop at C prevents the ribosomes from continuing from site A into the lysozyme cistron but ribosomal attachment site B is still blocked by base-pairing. This nuclease-treated mRNA is consequently incapable of directing lysozyme synthesis. By heat disaggregation, however, a small mRNA fragment is liberated which can be translated to yield lysozyme since ribosomal attachment site B is no longer masked by base-pairing.

Processing of mRNA?

Aggregation artefacts cannot explain the fact that the lysozyme mRNA activity extracted from T4-infected cells sediments with a definite peak at about 18S and a shoulder at about 13S (Fig. 4). Corresponding peaks occur under conditions which prevent aggregational artefacts (sedimentation in denaturing solvents or after removal of the stable RNA species) and in dimethyl sulphoxide about 39% of the mRNA activity sediments even faster than the main peak (1,700 nucleotides long, our unpublished results). Since all of the lysozyme mRNA activity is known by several criteria to come from a single structural gene⁹, we are forced to one of two possible conclusions. The first possibility is that the primary transcripts are themselves made in a variety of sizes, either because RNA polymerase transcribes lysozyme

mRNA from a number of different initiation points or because transcription stops at a number of 'partial' termination sites. The second possibility is that the lysozyme mRNA is transcribed as part of a very large molecule, at least 3,000 nucleotides long, and that all the smaller species are produced by endonucleolytic cleavages. Such cleavages could occur *in vivo*, in which case they represent natural mRNA processing steps, or they could occur during the extraction of the messenger (although this is carried out under conditions—sodium dodecyl sulphate, hot phenol—which should minimise nuclease action).

Of course the possibilities above are not mutually exclusive and each could play a role. Of the two we favour the second (processing type cleavage of a large precursor molecule) since the same size (13S and 18S) fragments can be obtained *in vitro* from the large mRNA molecules by RNase T₁ digestion.

Attempts to activate 5' lysozyme mRNA

As previously reported, mRNA extracted 5 min after T4 infection of *E. coli* (5' mRNA) is not competent for lysozyme synthesis in an *in vitro* protein synthesising system². Nevertheless, both hybridisation⁷ and transformation⁸ experiments have demonstrated that lysozyme mRNA is physically present at 5 min. The presence of factors early in infection which might inactivate early lysozyme mRNA and the possibility that the 'early' ribosomes are incompetent have been ruled out already². Our present experiments indicating that secondary structure can prevent translation of lysozyme mRNA support the idea that lysozyme mRNA might always be made in a form inactive due to its secondary structure. Late in infection such a lysozyme mRNA could be activated, perhaps by cleavage of a fragment which exposes the ribosomal attachment site or by modification of the structure.

With this in mind, we attempted the following experiments to activate 5' mRNA. To determine whether we could activate early lysozyme mRNA by adding some factor produced late in infection we replaced the S30 extract from uninfected *E. coli* with an S30 extract prepared from cells infected for 30 min with T4em74, a T4 mutant carrying a nonsense codon in the lysozyme *e* gene. No lysozyme synthesis was obtained with 5' mRNA even at the magnesium concentration which

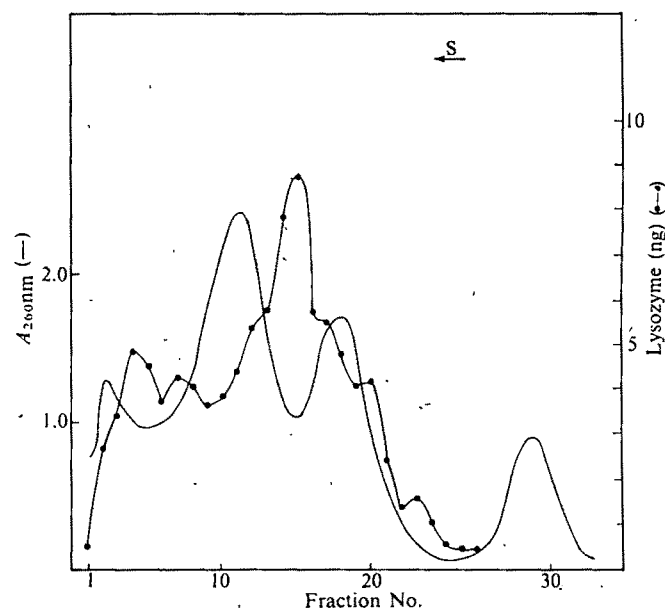


FIG. 4 Two mg RNA extracted with sodium dodecyl sulphate and hot phenol from *E. coli* infected with T4D¹ was layered onto a sucrose gradient, as described in Fig. 2. The recovery of activity was 96%. —, A_{260 nm}; ● — ●, ng lysozyme synthesised.

gave optimal synthesis with the late mRNA control. Attempts were also made to activate the early lysozyme mRNA by carrying out the *in vitro* synthesis at elevated temperatures or by limited digestion of the early mRNA with RNase T1. The RNase T1-treated material was tested both with and without a subsequent heat disaggregation. No evidence for activation of 5' lysozyme mRNA was obtained even though the controls carried out with 25' mRNA indicated that we had succeeded in covering the optimal range of each one of these treatments.

Base-pairing in lysozyme mRNA non-random

It has long been established that the mRNA from the RNA bacteriophages is highly base-paired. Recent work with f2 and R17 RNA suggests that the secondary structure of these molecules is related to translational control^{9,10}. But the RNA phage genomes may be unlike other mRNA species since they are self-replicating and must be capable of folding to fit a spherical virus capsid: their secondary structure might be for instance, a special adaptation to facilitate encapsulation^{11,12}. For this reason we have examined bacteriophage T4 messenger RNAs. Since they are synthesised from a double stranded DNA genome their characteristics may be more nearly typical of other mRNA species.

Our previous experiments utilising several indirect methods (optical studies, partial digestion with single-strand specific nucleases, and sedimentation under denaturing and non-denaturing conditions) suggested that T4-specific mRNAs contain a considerable amount of base pairing (submitted for publication). But Fresco *et al.*¹³ have asserted that even random RNA sequences may contain as much as 60% base pairing. If the random base pairing structures they proposed are compared directly with those derived from direct sequencing of 16S rRNA^{14,15} it becomes immediately obvious that the random structures should have much lower melting temperatures, less cooperativity in their melting curves and greater sensitivity to single-strand-specific nucleases. Since our previous experiments show that T4 mRNAs are very similar to rRNA in all these regards, (and unlike

random copolymers, our data in preparation) it seems clear that chance base pairing cannot explain the secondary structure observed.

The idea that the secondary structure of lysozyme mRNA is highly ordered is further supported by our finding that nuclease treatment yields active fragments in discrete size classes. The mRNA molecules would be expected to exist in a large number of different configurations with similar stabilities if only random base pairing were involved. If so, nuclease digestion of exposed single stranded regions should not have given the discrete peaks of activity observed in Fig. 2c and e.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Difficulty of interstellar radio communication

IN 1959 Cocconi and Morrison¹ suggested that the hyperfine transition of atomic hydrogen at 1,420.405 MHz is the uniquely rational choice of frequency to use in attempts at interstellar communication. Since then other rational choices have been put forward: for example, the first or second overtones of this frequency^{2,3} or the frequency of one of the hydroxyl transitions at 1,612, 1,665, 1,667 and 1,720 MHz^{3,4}. Very recently Drake and Sagan⁵ have argued ingeniously in favour of yet two further frequencies. Unfortunately, none of the rational choices may now be treated with confidence as the correct one. This magnifies the difficulty of the problem.

By an understandable process of self-selection, writers on the subject of interstellar radio communication tend to be at

least cautiously optimistic. The view of the perhaps rashly pessimistic should not be allowed to suffer by default.

Following Drake and Sagan⁵ I shall for definiteness make what they refer to as "the rather optimistic assumption" that the number of stars n_s which must be searched is about 10^6 , which corresponds to the number of civilisations N in the Galaxy being about 10^5 and to the likely distance to the nearest civilisation being several hundred (say 300) light years. In the steady state⁶

$$N = RL \quad (1)$$

where R is the annual rate at which civilisations evolve and L is their average lifetime. It has been estimated³ that R may be as high as 10^{-1} yr^{-1} , in which case equation (1) may be written

$$N = 10^{-1}L \quad (2)$$

and for N to be 10^5 would require L to be 10^6 yr .

The probability of a transmitting radio telescope on a

distant planet (civilisation X) and a receiving radio telescope on Earth (civilisation E) being directed toward one another at corresponding times is minute. Sophisticated survey schemes may be envisaged but may be ignored in the context of an order of magnitude calculation.

To favour the feasibility of the civilisations making radio contact, I shall suppose that both X and E take their orbital motion around their parent star accurately into account, and I shall suppose further that the presumably technically very much more advanced X measures the radial velocity of every star in the survey to within $\pm 0.01 \text{ km s}^{-1}$, as seems possible⁷, and also takes this into account. At the frequency of the hyperfine line of atomic hydrogen (which for definiteness I shall use for illustrative purposes) the uncertainty in the Doppler shift would then be about 10^2 Hz . To keep the number of channels as few as possible I shall suppose that X transmits on a band of this width. The power radiated would have to be high. For example, if the dishes of both radio telescopes were 300 m in diameter, some 10 MW would be needed to reach a distance of 300 light years with a strength equal to sky noise at a temperature as low as 10 K. Finally, to favour the feasibility of the venture under consideration yet again, I shall suppose that E guesses X's general procedure (and is content to use a single channel).

The times required for X and E to survey n_s stars systematically are respectively

$$J(X) = n_s n_c \tau(X) \quad (3)$$

and

$$J(E) = n_s \tau(E) \quad (4)$$

where n_c is the number of channels regarded as rational choices and where $\tau(X)$ and $\tau(E)$ are the X and E times per star per channel.

Sending a significant signal takes longer than detecting the existence of a signal, so

$$\tau(X) > \tau(E) \quad (5)$$

and hence

$$J(X) > J(E) \quad (6)$$

Accepting equations (5) and (6) it is a straightforward task to prove that during any complete survey of the n_s stars by X the probability that two dedicated radio telescopes would be directed towards one another at corresponding times when using the same channel is

$$P = \tau(X)/J(E) \quad (7)$$

provided

$$\tau(X) < J(E) \quad (8)$$

Omitting the time of passage between X and E, the time before radio contact is likely to be established is therefore around

$$T = J(X)/P \quad (9)$$

$$= n_s^2 n_c \tau(E) \quad (10)$$

Subject to condition (8) being satisfied, T is independent of $\tau(X)$ so that quite an extended message could in principle be transmitted.

In view of the suggestions referred to in the first paragraph, I think it not unreasonable to assign the value 4 to n_c , in which case (10) may conveniently be written

$$T = 10^5 \tau(E) \text{ yr} \quad (11)$$

where $\tau(E)$, which of course includes the time lost in changing from one star to the next, is measured in s. It was the

practice to observe a band 100 Hz wide for 60 s in Project Ozma. Though $\tau(E)$ could certainly be made much briefer than this, it is apparent from equation (11) that T is a depressingly long time. The position would naturally be eased if X and E were each sufficiently concerned to use a number of dedicated radio telescopes. Some adverse factors not mentioned here have to be taken into consideration (see ref. 8).

Agreeing with the penultimate sentence of the previous paragraph the referee has argued that there is no real problem because X is likely to use as many as 10^5 dedicated radio telescopes, the cost being of little moment owing to X's highly advanced technology. This is indeed conceivable (though if the population of X were, say, 10,000 million it would correspond to one dedicated radio telescope per hundred thousand inhabitants). I think, however, that nothing can be said with confidence about the true wealth of X, which is determined by many complex factors such as the balance between the effects of technical progress and depletion of natural resources. Again I think it is open to question whether X's interest in galactic anthropogeny would be deep and long lasting.

An entirely different time is now of relevance. How long would a search project be continued in the absence of success? I am sceptical that it would be continued for even a century but adopting a very favourable view I shall suppose that it would continue for 500 yr. Furthermore, I shall suppose that the complete failure of one search project would not prevent another being initiated at some later date. Repeated failures would make fresh starts ever more difficult and 10^4 yr seems to me a very generous upper limit to the average total duration of the searches, that is to the average lifetime of the civilisation in communicative phases. This increases the effective extent of our isolation.

Referring to equation (2) it may be seen that a consequence is that the number of communicative civilisations in the Galaxy is at most 10^3 , from which it follows that the likely distance to the nearest is at least several thousand light years. Perhaps most serious, the value of the right side of formula (11) for the time before contact between a pair of dedicated radio telescopes is likely to be made becomes $10^7 \tau(E)$ years or, more probably, very much longer (some of the favourable suppositions made in the derivation of the formula being now untenable). The prospect is unpromising.

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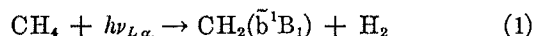
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CH₄ and CH₂ in comets

MANY of the simple radicals observed in cometary spectra, for example OH and NH, probably originate as dissociation products of more stable parent molecules such as H₂O and NH₃. Ip and Mendis¹ have recently discussed the possibilities of observing H₂O in Comet Kohoutek (1973f). If CH₄ exists in significant abundance in comets, it could be a parent of observed CH and CH⁺. It is difficult to observe CH₄ directly but a rather specific though indirect means of detecting its presence is suggested.

Dissociation of CH₄ by monochromatic photons at the wavelength of hydrogen L α (1,216 Å) results in



(see ref. 2). Fluorescence, resulting from the radiative decay of the excited CH₂ by emission in the $\tilde{b}^1B_1 - \tilde{a}^1A_1$ system, accompanies this process. Hydrogen L α is the strongest emission line in the solar spectrum and its intensity at any point in the orbit of a comet can be calculated. A search for the lines of CH₂ ($\tilde{b}^1B_1 - \tilde{a}^1A_1$) lying between wavelengths of 5,000 Å and 9,000 Å (refs. 3 and 4) may be a useful, specific probe for both CH₄ and CH₃.

It is safe to assume that there is no other process capable of exciting the \tilde{b}^1B_1 state of CH₂ efficiently, and that the state is unlikely to be deexcited by collision before radiating. The total emissivity in lines of CH₂, $E(\text{CH}_2)$, is related to the concentration (cm⁻³) of CH₄, $n(\text{CH}_4)$ (cm⁻³), and to the solar L α flux, $\Phi_{L\alpha}$, according to

$$E(\text{CH}_2) = \Phi_{L\alpha} n(\text{CH}_4) \sigma(\text{CH}_4) \text{ photons cm}^{-2} \text{ s}^{-1}$$

where $\sigma(\text{CH}_4)$ is the cross section of process (1), estimated to have a maximum value of about 1×10^{-20} cm² (ref. 2). At a distance of 1.0 AU from the Sun, $\Phi_{L\alpha} \cong 3 \times 10^{11}$ photons cm⁻² s⁻¹ (ref. 5), and therefore

$$E(\text{CH}_2) \cong 3 \times 10^{-9} n(\text{CH}_4) R_{sc}^{-2} \text{ photons cm}^{-2} \text{ s}^{-1}$$

where R_{sc} is the distance (AU) of the comet from the Sun. The total flux in the lines of the $\tilde{b}^1B_1 - \tilde{a}^1A_1$ system of CH₂ received at the Earth is given by

$$\Phi(\text{CH}_2) \cong 8 \times 10^{-10} R_{sc}^{-2}$$

$$\int n(\text{CH}_4) dl \left(\frac{R_{coma}}{R_{sc}} \right)^2 \text{ photons cm}^{-2} \text{ s}^{-1}$$

The integration is along the path length over which CH₄ is in gaseous form in the coma, R_{coma} is an average size describing the region of emission and R_{sc} is the Earth-comet distance.

For Comet Kohoutek (1973f) near perihelion, $R_{sc} \cong 0.15$ AU, $R_{coma} \gtrsim 10^{11}$ cm and $R_{sc} \cong 1.0$ AU = 1.5×10^{13} cm, and therefore a flux at the Earth in the CH₂ lines

$$\Phi(\text{CH}_2) = 2 \times 10^{-12} \int n(\text{CH}_4) dl \text{ photons cm}^{-2} \text{ s}^{-1}$$

An effective column density $\int n(\text{CH}_4) dl$ of 10^{15} cm⁻² should produce detectable CH₂ emission even if it is distributed over 100 lines of roughly equal strength: approximately 2×10^3 photons s⁻¹ per line, assuming a 60 inch telescope with a spectrometer or spectrograph such that the quantum efficiency for the complete system is 0.01. The vacuum wavenumbers of the CH₂ lines which have been observed in absorption are given by Herzberg and Johns⁴.

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Sixth radio outburst of Cygnus X-3

FIVE large outbursts of radio emission from Cygnus X-3 have been reported, (refs 1-3 and V. A. Hughes, unpublished). We present here observations of a sixth, which occurred in June and July 1973. The observations were made using the 46-m telescope of the Algonquin Radio Observatory at 10.6 GHz (bandwidth ~ 100 MHz, system temperature ~ 120 K). A total of 60 flux density measurements, each providing 7 min of integration time on the source, were made during the period June 25 to July 6, 1973. These observations were taken at sporadic intervals during our programme of measuring early-type emission-line stars, and the observing technique described in ref. 4 was used.

In Table 1 and Fig. 1 we give the measured flux density values for Cygnus X-3. Allowance has been made for the effects of confusion, using data provided by P. C. Gregory which had been derived from observations of the first recorded outburst. The error bars are standard deviations representing the combined effect of random noise (which in many cases was large, due to poor weather conditions), variations in calibration level, and several smaller factors such as the uncertainty due to confusion effects. The flux density scale is based on 3C123 = 7.92 f.u., and 3C274 = 37.1 f.u.

The decay of the sixth outburst was exponential. An exponential decay was also noted for burst 1 (ref. 5), and our analysis of the published data for the other outbursts

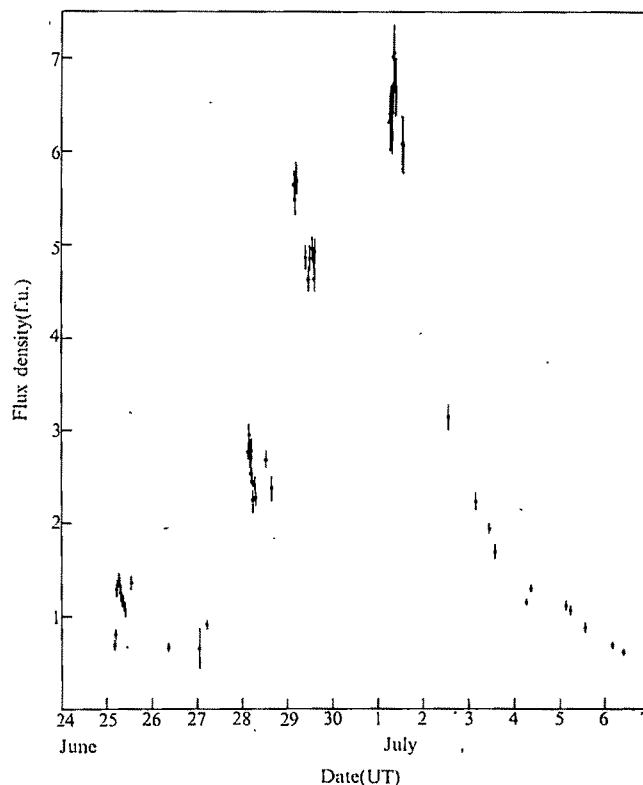


FIG. 1 Measured flux densities of Cygnus X-3 at 10.6 GHz during the sixth outburst.

indicates similar behaviour, suggesting that an exponential decay is a general characteristic of Cygnus X-3 radio bursts. This is clearly incompatible with source models involving simple adiabatic expansion, which predicts a power-law decay⁶.

We have developed an alternative model for the Cygnus X-3 radio source. We propose that ambient gas affects the radiation from the synchrotron source in two ways: (i) A high density gas occupies part of the path traversed by the synchrotron-emitting electrons, causing them to lose energy primarily by bremsstrahlung losses. This would explain the exponential decay of the radio bursts, as was suggested⁵ for the first outburst. (ii) Each outburst is accompanied by an expanding cloud of ionised gas which surrounds the synchrotron source. If the cloud is initially optically thick at a particular frequency, then the synchrotron source will be obscured. As the gas expands, the decreasing

free-free optical depth will result in an increase of the observed intensity. Further the observed outburst will be progressively delayed at lower frequencies. In that case, the form of the rise would depend primarily on the characteristics of the expanding cloud. But if the cloud is optically thin at a particular frequency, then the synchrotron source will be unobscured, and the outburst will be observed simultaneously at all higher frequencies. In that case the form of the rise depends directly on particle injection in the synchrotron source.

This model explains several features of the observed bursts: the exponential decay, the time delay at lower frequencies of some bursts, and the simultaneous rise over a wide range of frequencies of other bursts.

It is interesting that analyses of Cygnus X-3 based on its radio spectrum⁷ and X-ray characteristics⁸ have also led to models which require the presence of a thermal absorbing cloud.

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TABLE 1 Measured flux densities of Cygnus X-3 at 10.6 GHz during the sixth outburst

Date	Time (UT)	Flux density (f. u.)	Standard deviation (f. u.)
June 25	0417	0.678	0.051
	0447	0.799	0.057
	0513	1.284	0.083
	0548	1.330	0.085
	0618	1.389	0.088
	0626	1.339	0.085
	0639	1.246	0.081
	0738	1.172	0.076
	0813	1.135	0.074
	0936	1.059	0.071
	1248	1.359	0.087
June 26	0842	0.671	0.050
June 27	0101	0.65	0.22
	0705	0.911	0.042
June 28	0239	2.764	0.081
	0249	2.96	0.11
	0259	2.815	0.082
	0310	2.709	0.079
	0336	2.77	0.12
	0403	2.527	0.096
	0414	2.45	0.11
	0425	2.24	0.13
	0441	2.424	0.072
	0549	2.263	0.068
	1207	2.691	0.079
	1456	2.38	0.13
June 29	0309	5.65	0.15
	0320	5.48	0.15
	0330	5.73	0.16
	0340	5.70	0.15
	0920	4.87	0.13
	1038	4.63	0.13
	1048	4.86	0.13
	1234	4.96	0.13
	1248	4.84	0.13
	1256	4.64	0.13
	1304	4.93	0.13
July 1	0603	6.32	0.30
	0609	6.40	0.30
	0619	6.38	0.30
	0632	6.27	0.30
	0645	6.44	0.30
	0823	6.70	0.32
	0834	7.02	0.33
	0815	7.06	0.33
	0842	6.66	0.31
	0930	6.70	0.32
	1253	6.09	0.29
July 2	1331	6.07	0.29
July 3	1257	3.15	0.15
	0330	2.23	0.11
July 4	1100	1.942	0.056
	1403	1.686	0.089
	0641	1.153	0.039
July 5	0911	1.295	0.042
	0305	1.118	0.040
	0520	1.055	0.042
July 6	1340	0.866	0.064
	0402	0.692	0.034
	0950	0.612	0.033

Distribution of late Pleistocene aeolian deposits in eastern and southern England

THERE has been much recent interest in the possible existence of widespread aeolian deposits in Britain. In southern England brickearths at Pegwell Bay¹⁻³ (TR36) and elsewhere⁴ have been interpreted, although without mineralogical confirmation, as extensions of European loess. Silt, in excess of that which could be derived from weathering of the substrata and generally considered loessial, is found in many British soils⁵⁻¹⁰ but it is not ubiquitous. For example, although it occurs in soils on Chalky Boulder Clay in Essex¹¹, on the same till in central East Anglia and on the Chalk of the Breckland and north-west Norfolk it is replaced by sand.

The lithology, and hence likely weathering products, of the Chalky Boulder Clay are very uniform over a large area of eastern England¹². The till thus provided a unique substratum on which to survey the distribution of sandy and silty surface deposits. The patterns were later traced onto

TABLE 1 Soil provinces in East Anglia and East Midlands

Province		I	II	III	IV	
Type of composition curve		Unimodal	Bimodal	Unimodal	Bimodal	
Peak particle diameters						
Sand grade	Range		120-390	150-370	150-350	
	Mean		201	219	236	
	Standard error		33.5	37.8	55.2	
	Mode		210	210	210	
Silt grade	Range	26-68	16-48		28-50	
	Mean	48.8	31.8		37.2	
	Standard error		0.50		0.75	
	Mode		30		37	
No. of samples		5	141	102	46	Total 294

other formations and into other areas. The survey is summarised here and will be described in detail elsewhere.

East Anglia and east Midlands: The top 4 cm of the soil were sampled at least once in every 10 km grid square containing Chalky Boulder Clay in East Anglia and, at a slightly lower density, in the East Midlands with appropriate replication (190 samples). To minimise effects of solifluxion or downwash each sample was taken from the top of an interfluvium, normally the highest point in the square.

Mechanical analysis of the carbonate-free fraction < 2 mm of every sample showed that the soils were unlike any conceivable weathering residue of the under-lying Chalky Boulder Clay and could be grouped into four provinces defined by the unimodal or bimodal form of the composition curves and by peak particle diameters (Fig. 1 and Table 1). Soils of province I are clearly enriched in silt, those of III in sand and those of II and IV in silt and sand. Once recognised on the lithologically uniform till the provinces were traced across interfluvium on Cretaceous sands, Chalk, Tertiaries, Norwich Brickearth and the March and Observatory Gravels of Cambridgeshire; and onto flat sites on the Terrace

Gravels of the Ouse, Cam and Lark (104 samples). Their distribution is shown in Fig. 2; they transgress geological boundaries, are unrelated to land-form or altitude and, except between I and II, are sharply separated.

There is a tendency, shown by differences between the means and modes, for peak diameters in the sand grades to decrease southwards, although ranges are similar (Table 1); no east-west trends have been observed. Peak diameters in the silt grades show no consistent geographical relationships.

Heavy mineral counts on the fraction 105 to 63 μm (65 samples) showed that the soils of Province III are the least like the till (Table 2), being significantly different in total heavy minerals ($P < 1.0$) and in opaques, rutile, zircon, epidote, amphiboles and micas. Those of provinces II and IV differ significantly in opaques, zircon and micas, and those of I differ in garnet, rutile, zircon and micas, although only five samples have been examined. Apart from the absence of mica in III there are no qualitative differences between the till and the various soils, suggesting that all the sand grades may have a common ultimate source. Quantitative differences could reflect derivation from different parts of that source or modifications during transport.

Stones in the topsoils cannot be compared directly with those in the till since chalk, the commonest erratic, dissolves in soil formation. But counts of 500 non-calcareous stones at each of 25 sites showed that there are no significant differences between the till and the soils. This suggests, though it does not prove, that surface drifts on the Boulder Clay are not merely the remnant of a later glaciation¹².

The only satisfactory explanation for all these observations is that thin silty and sandy drifts were added to the surface and incorporated by processes such as frost-heaving and biological mixing. Their wide persistence across differing substrata, their invariable occurrence on the highest interfluvium and, within each province, the uniformity of peak particle diameters (with values typical of blown sands and loess) support aeolian origins and argue against deposition by water, ice or ablation.

In province III, the peak diameters, low silt contents and absence of micas indicate that the soils are probably derived from blown sand more or less mixed with the substrata (Fig. 1c). Since the Breckland falls clearly in this province it now seems probable that the sandy-chalky drift and soils of that area are derived ultimately from blown sand rather than a local sandy till¹³.

In provinces I, II and IV, loess is the likely source of the widespread silt since heavy minerals in the fraction 63 to 20 μm are similar throughout. Province IV (46 sites) includes a smaller area (six sites) of 'cover loam' soils reported to contain loess from Devensian deposits¹⁴.

In provinces II and IV the bimodal composition must result from deposition of both sand and silt. Since peak particle diameters in the mineralogically similar sand fractions are comparable in II, III and IV, although with a small trend in mean values, these provinces are probably all parts of one blown sand complex. As pointed out below, the relationship to topography supports this conclusion. The

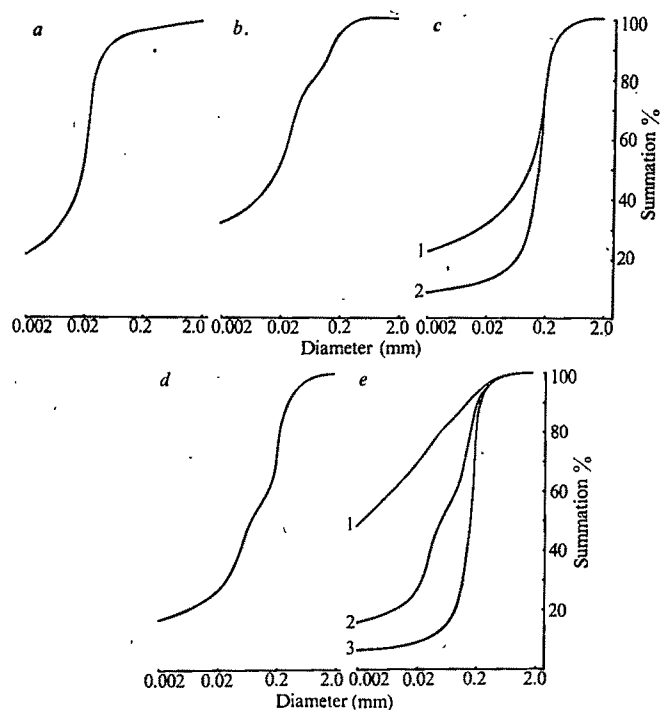


FIG. 1 Cumulative mechanical composition curves. *a*, *b*, Topsoils typical of provinces I and II respectively. *c*, Two topsoils typical of province III; 1, High-clay topsoil overlying and incorporating material from Chalky Boulder Clay; 2, Low-clay topsoil overlying the Chalk. *d*, Topsoil typical of province IV. *e*, Section at Great Blakenham, Suffolk; 1, Chalky Boulder Clay at depth of 4.5 m; 2, Topsoil, showing addition of silt and sand; 3, Sand pocket very low in silt at 1.0 m.

TABLE 2 Mineralogy of fractions 105–63 μm

Lithology Province	East Anglia and East Midlands										Lincolnshire		Kent	
	Boulder Clay I, II, III & IV		I		II		III		Topsoils		IV		III	IIa
	Mean	s.e.	Mean	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean
Heavy Minerals*	2.54	0.254	1.20	1.50	0.199	1.57	0.160	0.99	0.152	0.75	0.094	1.88		
P					<1.0			<1.0	<0.2		<0.2			
Opagues†	85.0	1.08	87	75.2	2.30	72.6	1.94	58.1	3.46	72.2	3.21	86		
Rutile group†	2.9	0.65	10	4.6	0.65	8.1	0.66	5.1	1.20	11.1	1.14	12		
Staurolite	3.3	1.08	4	2.7	0.38	2.6	0.29	2.3	0.33	1.8	0.54	5		
Tourmaline	5.5	0.84	9	8.9	1.08	9.4	0.97	5.9	0.81	7.6	1.49	6		
Garnet group	21.0	1.40	12	26.8	1.33	24.1	1.15	20.2	2.10	17.5	1.87	8		
Zircon	9.5	0.92	25	15.5	1.25	36.9	1.88	17.8	2.99	40.6	3.60	47		
Epidote group	19.7	1.44	23	19.2	1.17	12.6	0.92	24.3	2.29	7.8	1.31	5		
Mica group	12.3	2.24	2	1.1	0.39	0		3.4	2.45	0		0		
Amphibole group	23.8	1.78	15	21.0	1.44	6.9	0.56	20.3	2.34	10.4	1.56	11		
Others	0.4	0.35	0.2	0.4	0.16	0.1	0.08	0.3	0.13	2.5	0.70	3		
No. of samples	33		5	25		29		9		17		3		

* Percentage by weight of fraction 105 to 63 μm e.s.d.

P is the measure of significance of the difference of the heavy mineral weight percentage from the Chalky Boulder Clay.

† Percentage by number of total heavy minerals.

‡ Percentage by number of non-opaque heavy minerals.

suggestion that the sand component in the cover loam soils was derived from subjacent deposits¹⁴ may be locally true but is not generally valid as the provinces cross some formations which could not provide such material.

Lincolnshire and Yorkshire: Mechanical analysis of the topsoils from 55 interfluvial sites in Lincolnshire and east Yorkshire showed that province III extends beyond the Fens and continues across the Humber. The heavy minerals in these areas and those of III in East Anglia are not significantly different (Table 2). Just as the Breckland seems to be a thick accumulation of the sands of this province in Norfolk, so the 'cover sands' south of Scunthorpe (SE91) appear also to be mechanically and mineralogically part of it rather than a purely local deposit redistributed from the Trias¹⁵.

The boundary between provinces II and III was traced as far as Sleaford (TF04) (Fig. 2). On the east side of III silt accumulations were found on the Lincolnshire and Yorkshire Wolds (Va and Vb). East of the Wolds other Devensian deposits and alluvium make interpretation difficult but there was no evidence for any extension of province IV at any of the fourteen sites examined.

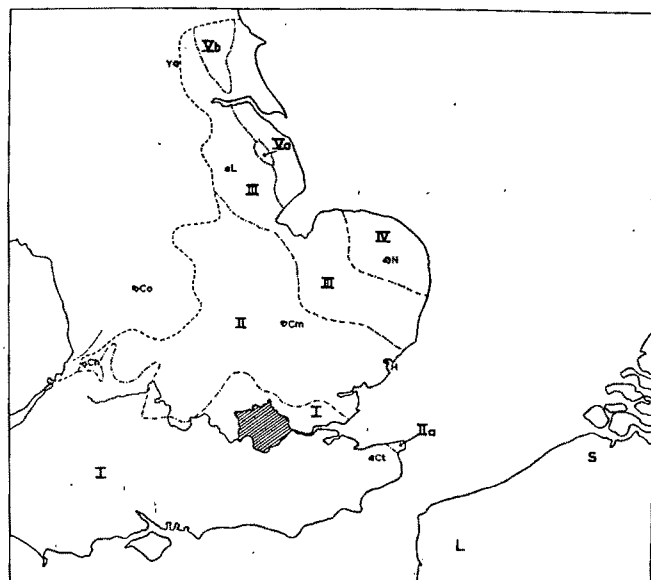


FIG. 2 Distribution of provinces in eastern and southern England. — — —, Province boundaries; — — —, present limits of sampling. Ch, Cheltenham; H, Harwich; Cm, Cambridge; L, Lincoln; Co, Coventry; N, Norwich; Ct, Canterbury; Y, York; S, cover sand zone in Belgium and the Netherlands.

Midlands and southern England: A further 252 interfluvial sites were sampled, on the Boulder Clay and on other formations suitable for the detection of extraneous material. These were granites, serpentine and gabbro; Devonian, Carboniferous and Jurassic limestones; Jurassic clays, the Lower Greensand, Chalk, Clay-with-flints and various gravels. All areas previously shown to contain loessial soils⁵⁻⁹ were included. Sampling density was lower than in East Anglia and unsuitable substrata excluded many areas, particularly in southern England (Fig. 3).

Mechanical analysis of all samples showed that the boundary between provinces I and II, although not clearly defined, extends westwards to near Cheltenham (SO92) and south-eastwards into Thanet, where there is a small area of soils (IIa) mechanically similar to those of II but with quantitative differences in mineralogy (Figs 2 and 3, and Table 2).

In province I, although their extraneous origin was sometimes uncertain, accumulations of silt with peak diameters ranging from 16 to 50 μm were found in 177 samples (Fig. 3). The conclusion that this persistent silt is loess is supported by heavy mineral compositions of the fractions 105 to 63 μm and 63 to 20 μm . The former is generally closely similar to the same grade in the substratum but the latter is fairly constant over the whole area (Table 3).

Within province I there are locally a few bimodal soils. Although some of their sand fractions are clearly derived from substrata, such as Lower Greensand or remnants of Reading Beds on Chalk⁹, others seem to be definitely aeolian. More intensive sampling might allow this province to be sub-divided.

Peak diameters for the sand fractions of some soils of provinces II, III and IV lie above 210 μm ('coarse' soils), the remainder below ('fine'). The frequency of occurrence of 'fine' soils is unrelated to province boundaries or geographical coordinates but shows a statistically significant tendency to increase with altitude (Table 4).

In East Anglia the long axis of the area of high altitude 'fine' soils lies perpendicular to the boundary between II and III suggesting that these contain the same blown sand complex which has been similarly affected by topography in both of them.

Since there is no relationship between silt grade peak diameter and either geographical position or altitude, it is possible that, whereas during saltation finer sand could better surmount gradients than coarse, silt deposited from suspension was more influenced by local factors such as air turbulence or vegetation.

In East Anglia, the sands of provinces II, III and IV are frequently involuted into the Boulder Clay, presumably by

TABLE 3 Heavy minerals in soils from southern England and northern France

Locality Substratum	Seworgan Cornwall SW7131 Granite	Brixham Devon SX9456 M. Devonian Limestone	Cannington Somerset ST2440 Carboniferous Limestone (d ₂)	Wellow Somerset ST7459 Gt Oolite Limestone	Chalton Hants SU7315 U. Chalk	Peter's Green Herts TL1419 Chalky Boulder Clay	Cliffsend Kent TR3564 Brickearth	Hucqueliers France Clay-with- flints over Chalk	Forêt d'Eperlecques France Clay-with- flints over Chalk
Opagues*	27	58	100 72	51 35	100 61	84 58	86 60	100 56	100 63
Rutile									
group†	<1	7	11	8	4	10 8	2 10	8	10
Staurolite	4	<1			<1	2 <1	7 1		<1
Tourmaline	1	<1	1	<1	1	5 <1	1	<1	1
Garnet									
group	4	8	6	10	4	5	7 7	10	42
Zircon	17	24	25	17	19	49 27	41 19	17	7
Epidote									
group	18	44	43	50	39	12 42	26 39	50	20
Mica									
group	99‡	50‡	<1	2	2	2 2	2 2	<1	1
Amphibole									
group	17	15	11	7	30	20 14	13 30	14	19
Others	1	3	<1	<1	<1	<1	2 <1	<1	1
Barite			100 §	30 §					

* Percentage by number of total heavy minerals

† Percentage by number of non-opaque heavy minerals

‡ Note the specially high proportion of micas in both fractions of granite soils

§ Barite is locally present as veins in the limestone in this area

The first column under each locality is for the fraction 105 to 63 μ m, the second for the fraction 63 to 20 μ m.

cryoturbation. Sections along the margin of the Breckland show that this was contemporary with that producing the well-known patterned ground¹³ which, being closely related to present topography, is generally regarded as Devensian. The sands must thus be at least as old as the last major periglacialiation in the area. But topsoils typical of Provinces II and III have been traced onto the March and Observatory Gravels and all three Terrace Gravels of the Ouse, Cam and Lark. The youngest of these, the First Terrace¹⁶, has been dated at $19,500 \pm 650$ BP (ref. 17) suggesting a probable maximum age for the sand.

In contrast to the sands, no interfluvial sites are known in East Anglia at which the silt has suffered periglacial disturbance and at Great Blakenham, Suffolk (TM103503) it is clearly younger than the sand: the soil here is bimodal but with increasing depth into the weathered till it becomes more sandy and there are deep sand pockets devoid of silt (Fig. 1e). One may deduce that the sand arrived first, was incorporated by cryoturbation and then covered by silt which was mixed by biological agencies to give the present sandy-silty topsoil. This is the most likely origin of all the bimodal soils for it is difficult to envisage any mechanism by which sand and silt could arrive simultaneously. Explanations for the distribution of provinces and the sharpness of most boundaries can so far be only speculative.

There is further evidence that the interfluvial silts in the south-east are relatively late. In the present study they have been traced by mechanical and mineralogical analysis across interfluvial in Kent into the top of the brickearth at Pegwell

Bay which lies above the highest frost structures^{1,2}. The survival of silt on exposed scarp crests on the South Downs and Yorkshire Wolds also suggests at least some deposition after intense solifluxion ceased⁹. No valid correlations have yet been made between the interfluvial silts and valley deposits in southern England reported as loessial^{4,18,10}.

TABLE 4 Peak particle diameter in relation to altitude

Altitude Feet OD	No. of samples 'Coarse' type	No. of samples 'Fine' type	P
0-300 (0-90 m)	162	117	<0.1
300-600 (90-180 m)	28	56	<0.1
600-900 (180-270 m)	1	13	<0.1

So it seems that the sands of provinces II, III and IV originally arrived before or during the last intense periglacialiation in the Devensian and the silts of I, II and IV later. It is difficult to accept that the latter could belong to the penultimate glaciation as has been suggested for some of the valley brickearths⁴.

Provinces I and III show some geographical relation to the zones of loess and cover sands established in Europe^{20,21} (Fig. 2). Preliminary mechanical analyses and heavy mineral counts confirm continuity between the silts of province I and loess on the Chalk of northern France (Table 3). But because of the general thinness and reworking of the sands of provinces II, III and IV, no valid stratigraphical correlation can yet be made with the thick series of cover sands in Holland and Belgium. This is being studied further.

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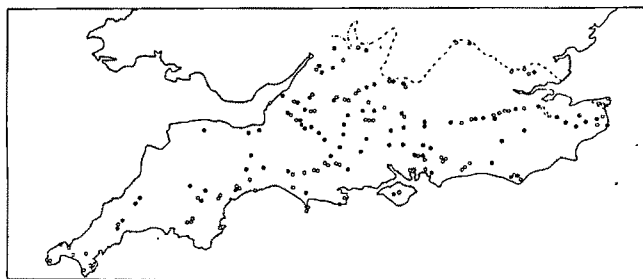


FIG. 3 Soil sampling sites defining province I, and province IIa in the Isle of Thanet. Each circle represents the site of one or more samples. (In the complex area east of Cheltenham sites are too numerous to show individually).

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Geophysical evidence for the intersection of the St Paul, Cape Palmas and Grand Cess fracture zones with the continental margin of Liberia, West Africa

PUBLISHED reconstructions of Gondwana continent¹ (Fig. 1a) show a gap in fit near the junction of the Americas and Africa. To study this critical area, the Unitedge I made geophysical measurements and collected rock samples across the continental margin of Liberia (USGS-IDOE cruise leg 5) in November 1971. Figure 1b indicates the location of the 5,400 km of ship track on a generalised bathymetric map². We shall discuss the data in detail elsewhere. Here we present the evidence for the existence of three fracture zones, two of which have not been reported previously, intersecting the continental margin at the north end of the South Atlantic, which remained closed probably until Cretaceous time. We suggest that Precambrian structures on the African continent controlled the location of these fracture zones. Figure 1c compares gravity and magnetic profiles and interpretations of the seismic profiles for three selected lines (27, 30 and 34) crossing the Grand Cess, Cape Palmas and St Paul fracture zones, respectively.

The bathymetric data (Fig. 1b) indicate that the north-west section of the continental margin of Liberia is narrow, the slope is moderately rough and the average gradient is about 3° (ref. 2). South-eastwards to about 9°30'W (about line 22) the slope gradually becomes smooth and wider and the average gradient is about 1.8°, which suggests depositional outbuilding. The topography of the area southeast of about line 22 reflects the effects of the inferred three fracture zones (the Grand Cess, Cape Palmas and St Paul fracture zones), where locations as indicated in Fig. 1b are interpreted from the magnetic and gravity data. Figures 2 and 3 show the magnetic and gravity profiles normal to the coast and indicate the anomaly correlations over the inferred fracture zones. The anomalies on lines 1 to 21, north-west of the fracture zones, are not discussed here but are shown for comparison. The St Paul fracture zone has been reported previously in

this area by others⁴⁻⁶. The comparison profiles shown for lines 27, 30 and 34 (Fig. 1c) are typical of the other lines crossed by the inferred fracture zones as indicated in Fig. 1b.

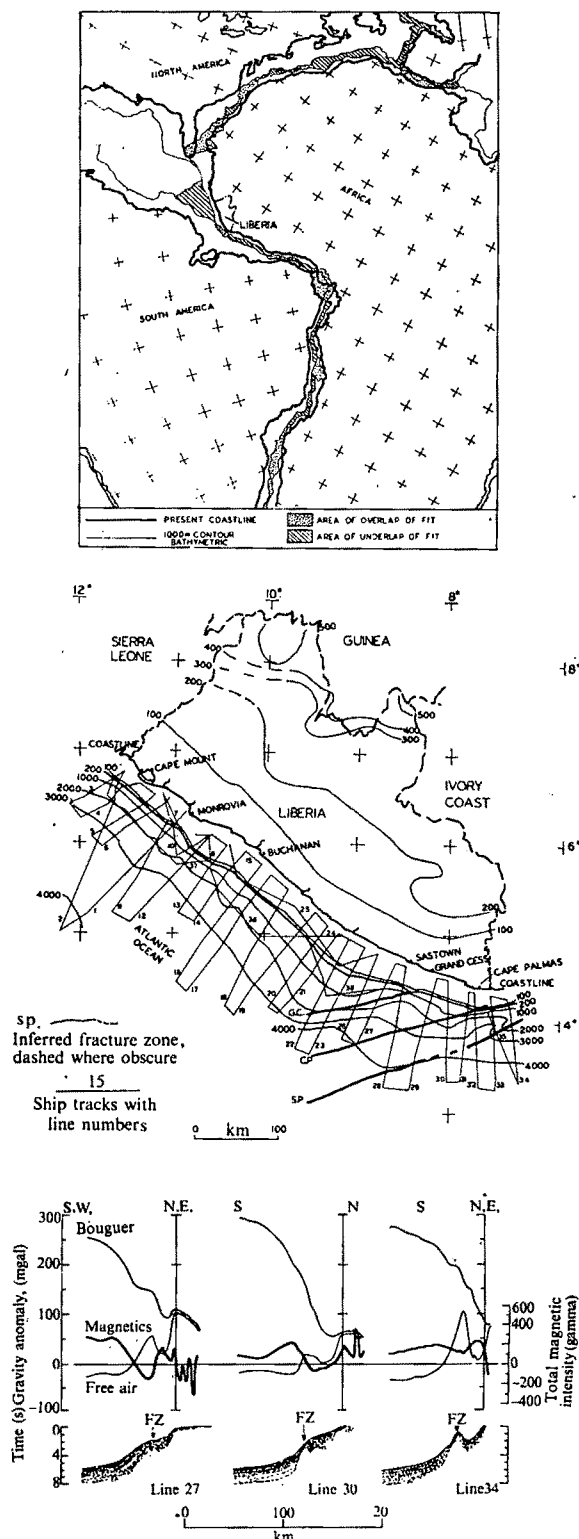


Fig. 1 a, Reconstruction showing predrift configuration of parts of continents around the Atlantic Ocean, taken from ref. 1. Note the good fit in the eastern third of Liberia and the poorer fit in the western two-thirds. Contours in metres. b, Index map showing generalised bathymetric and surface-elevation contours, as well as ship-track lines. Locations of Grand Cess, Cape Palmas, and St Paul fracture zones are indicated, as inferred from magnetic and gravity data. c, Geophysical profiles along lines 27, 30 and 34 crossing the Grand Cess, Cape Palmas and St Paul fracture zones, respectively. Seismic reflection interpretations are shown at bottom in two-way time.

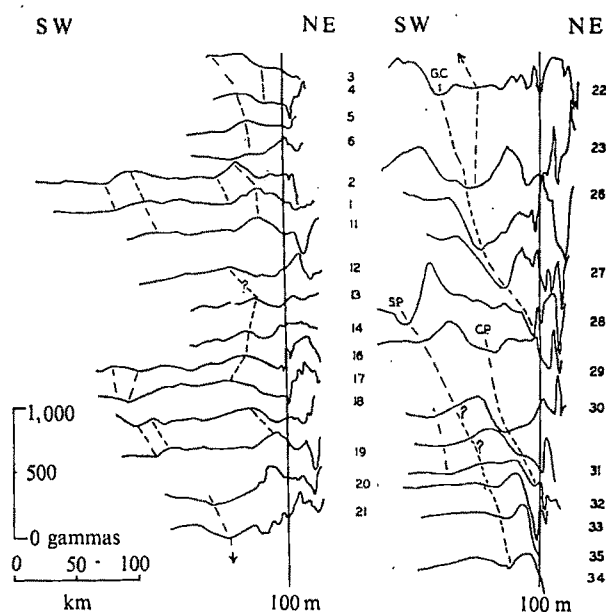


FIG. 2 Magnetic profiles for indicated track lines approximately normal to coast with IGRF removed. Suggested anomaly correlations are indicated. Tics indicate the inferred Grand Cess (G.C.), Cape Palmas (C.P.) and St Paul (S.P.) fracture zones are based on magnetic and gravity data.

We interpret the features labelled as the Cape Palmas and Grand Cess fracture zones on the basis of their similarity in geophysical character to the St Paul fracture zone. The extension of the Cape Palmas and St Paul fracture zones south-west of our ship tracks is based on earlier published magnetic data⁴.

Behrendt and Woterson^{7,8} presented data over the land and continental shelf and compiled a tectonic map based on these data and available geological information. Rock foliation and tectonic features trend east-northeastwards in the Eburnean province of south-east Liberia, as illustrated by aeromagnetic data (Fig. 2). These linear anomalies are asso-

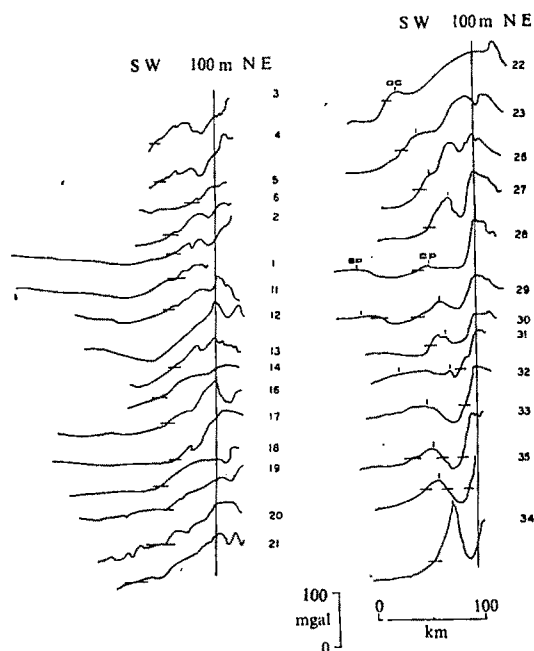


FIG. 3 Free air anomaly profiles for indicated track lines approximately normal to coast. Vertical bars indicate locations of Grand Cess (G.C.), Cape Palmas (C.P.) and St Paul (S.P.) fracture zones as inferred from magnetic and gravity data; horizontal bars indicate 0 mgal.

ciated with isoclinally folded and faulted paragneisses of Eburnean age ($\sim 2,000$ m.y.). Comparison of the trends of the fracture zones (Fig. 1b) with the trends of the magnetic anomalies of Fig. 4 shows that they are similar. The marine magnetic anomalies over the Grand Cess and Cape Palmas fracture zones (Fig. 1c) are continuous into the aeromagnetic anomalies of Fig. 2, although they are of longer wavelength because of greater water depths and depth of magnetic basement on the marine data. The magnetic anomaly associated with the St Paul fracture zone passes out of the surveyed area; this fracture zone intersects the margin of West Africa farther east⁴⁻⁶ (Fig. 1b). We do not imply, from this correlation, that the fracture zones and sources of magnetic anomalies on the continent are the same; the great discordance in ages between the Eburnean province (about 2,000 m.y.) and the time of rifting of Africa and South America during the Cretaceous precludes that. We interpret this coincidence in trends to indicate that the isoclinal folds and faults in the Eburnean province might have provided structural control for the location of the fracture zones developed at the time of rifting. In coastal Liberia the geological evidence of actual faulting that could definitely be said to be syntectonic with rifting is lacking, although work by Francheteau and Le Pichon⁵ suggests this possibility. A fault inferred from aeromagnetic data and confirmed by geologic mapping on land (A. H. Chidester, personal communication) occurs in the area where the Grand Cess fracture zone intersects the shelf. The tectonic map of Liberia⁹ also reveals several structures where the Grand Cess fracture zone intersects the shelf. We do not know the age of these features but they could be related to the fracture zones.

Though now buried, as indicated from the seismic data of Fig. 1c, the three fracture zones reported here have influenced subsequent deposition of sediments and the physiography of the slope and rise. Probably the broadening of the slope between line 13 and 22 (Fig. 1b) is related to the fracture zones, in the manner suggested by Francheteau and Le Pichon⁷ for the fracture zones in the Gulf of Guinea. This area, northwest of line 22, is the area of the gap in the Bullard *et al.*¹ reconstruction and was probably part of the continental margin earlier (at the time of the opening of the North Atlantic) than the area crossed by lines 23 to 35 (which originated at the time of the South Atlantic opening). At the scale of the entire South Atlantic, these fracture zones are very close together; in fact, they all lie easily between the 17° and 19° flow lines about Francheteau and Le Pichon's⁷ early pole of opening. The continuation of these inferred fracture zones into the similar northeast trending magnetic anomalies of the south-east Liberian continental shelf (Fig. 2) led us to conclude that the Precambrian isoclinally folded and faulted metamorphic terrane influenced their orientation and location at the time of opening of the South Atlantic.

Cochran⁹ showed several approximately north trending gravity and magnetic profiles west of our data (from about 15°W to 43°W). He identified the St Paul fracture zone crossing the Atlantic to the South American margin on the basis of its characteristic appearance on these profiles. The profiles on the African side (V2713 and V2604, in particular) and the profile nearest South America (V2502) are similar to those presented here. Just north of the anomaly associated with St Paul fracture zone on Cochran's data, are features we identify as associated with the Cape Palmas and Grand Cess fracture zones as defined here.

Probably the St Paul, Cape Palmas, and Grand Cess fracture zones are Cretaceous in age, at the intersection of the African continental margin, based on available information on the date of separation of Africa and South America (about 140 m.y. ago⁶). Previous interpretations^{7,8} that North America separated from Africa about 176 to 192 m.y. ago northwest of about line 22 (Fig. 1b) would require that the oceanic basement in this area be about 40 m.y. older than that to the

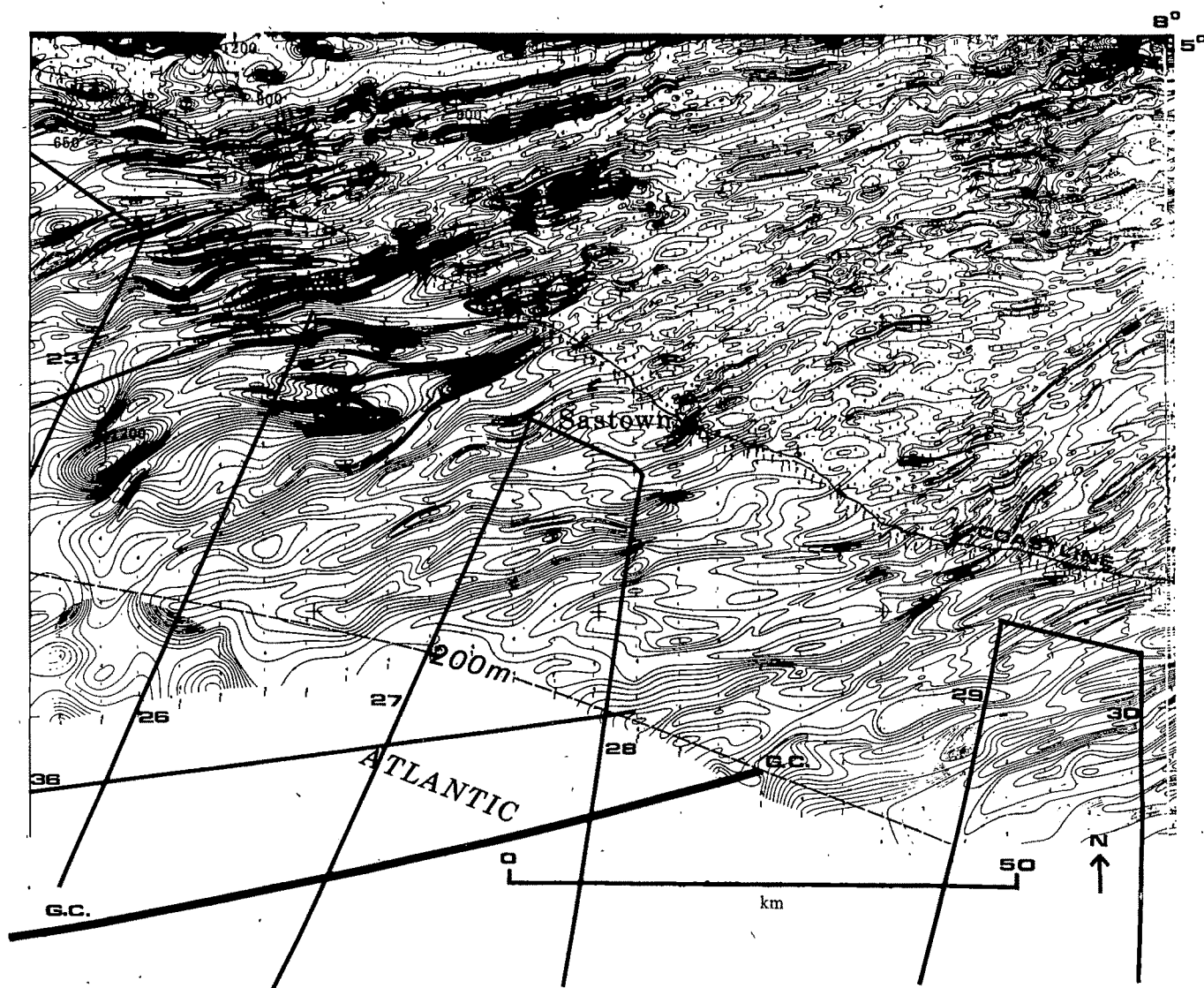


Fig. 4 Portion of aeromagnetic contour map in Sastown area with ship-track lines indicated. Contour interval is 10 and 50 gamma. Inferred location of Grand Cess fracture zone is indicated (G.C.). High amplitude anomalies are indicated in gammas.

south-east of line 22. If the Deep Sea Drilling Project (phase 3 leg 40) were to locate several holes in this area it would test this interpretation.

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Production of copper(I) halide emission spectra by MECA

THE introduction of copper(II) salts into most commonly used flames gives a green emission. Copper halides give a particularly intense emission, which is used in the well-known Beilstein test for halogens. The spectra from all copper salts, however, are very similar, and consist mainly of diffuse CuOH bands in the region 535–555 nm, a CuH band centred around 429 nm, CuO emission bands between 445 and 492 nm and a copper atom line at 327 nm (ref. 1, and Fig. 1). Copper(I) halide bands are also present when copper(II) halides are used, but are weak. The enhanced emission of the halides, therefore, arises mainly from their great volatility and consequent enhanced production of excited CuOH, CuO and CuH molecules, rather than greatly enhanced production of copper(I) halide emission. For this reason, pseudohalides such as cyanide and thiocyanate ions also give positive

Beilstein tests. The copper(I) chloride emission that has been observed in flames, especially when they are very fuel lean², is very insensitive for use in analysis.

Recently, we have developed a new, highly sensitive analytical flame technique named molecular emission cavity analysis (MECA)³. In this technique, the sample is placed in a cavity at the end of a steel rod, and the cavity is positioned in a hydrogen diffusion flame so that the flame gases flow almost vertically past the entrance to the cavity, with a small proportion entering the cavity. The flame also heats the exterior of the cavity. The emission characteristics of many materials placed in the cavity are appreciably different from those obtained by aspirating the sample solution into the flame. When microgram amounts of copper(II) chloride are placed in the cavity, or when traces of hydrochloric acid are introduced into a copper-plated cavity, the emission spectrum is very different from that obtained by aspirating an aqueous copper chloride solution into the flame. The spectra are compared in Fig. 1. Particularly noticeable is the much reduced intensity of the CuH, CuOH and CuO bands, and the greatly enhanced bands which can be attributed to copper(I) chloride emissions. The wavelengths of the main emission peaks are summarised in Table 1. The apparent triplet at the lowest wavelengths corresponds closely, in wavelength and relative intensity, with the main peaks of the D and E systems¹ of copper(I) chloride. Similarly the middle triplet corresponds with the B and C systems, and the longest wavelength triplet with the A system.

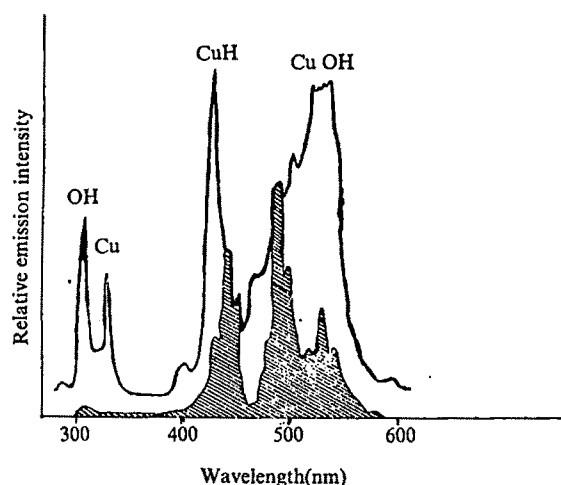


Fig. 1 Spectra of CuCl_2 in aqueous solution aspirated into $\text{H}_2\text{-N}_2$ diffusion flame (line drawing) and in the cavity (MECA) in the same flame (solid drawing).

Copper(II) bromide and iodide give significantly different spectra in the cavity. The wavelengths of the main peaks are summarised in Table 1. The copper bromide peaks at 480 nm and above correspond closely to those of the A system of copper(I) bromide, whereas the copper iodide peaks at 509 nm and above correlate well with those of the A system of copper(I) iodide. In all instances, the peaks at 437 nm do not correspond to CuH emissions, which would seem to be the only other possible emitters in this wavelength range.

The emissions can be used to detect nanogram amounts of halides. Other copper salts, such as copper(II) sulphate and nitrate, give no emission in the cavity.

Small amounts of tin(II) chloride or bromide in the cavity produce broad emission bands, with peaks at 440 and 460 nm, and 460, 480, 490 and 495 nm respectively. These do not correspond to the well characterised red SnH and blue SnO emissions but appear to be similar, however, to

TABLE 1 Wavelengths (nm) of major peaks in MECA spectra

CuCl	CuBr	CuI
425 (4)	437	437
437 (8)		
442 (5)		
475 (4)		
488 (10)*		
497 (6)		
515 (3)	480	509-511*
527 (5)	488*	514
539 (3)	496	520
	503	531

* most intense peak.
(relative intensities in parenthesis)

the blue or blue-green emission that appears just at the base of a hydrogen diffusion flame when the tin halide is aspirated, and is therefore probably tin halide emission. No spectral measurements of tin halide emissions in flames, however, have been reported. Similar emissions are obtained from halides of indium, lead, cobalt, cadmium and germanium.

The reason for enhanced halide emission from the cavity has not been completely established. It would seem to result, however, from the limited radical concentration in the flame gases within the cavity, which reduce the formation of non-halide copper species, and the relatively cool cavity surface, which enhances the emission of the emitting species as a result of the Salet phenomenon⁴.

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Teardrop shapes for small bubbles and drops moving through Newtonian liquids

In each of the previous observations¹⁻¹¹ of bubbles and drops with pointed tails the continuous liquid has had non-Newtonian characteristics. For Newtonian liquids, the shapes of drops and bubbles have been generally reported to pass smoothly from spherical to oblate ellipsoidal with increasing volume. Here we report observations of teardrop shapes for bubbles and drops in Newtonian liquids over a narrow range of sizes.

The experiments were done in a plexiglass column of cross section 39×32 cm and height 117 cm. The continuous liquid was an aqueous sucrose solution of density 1.393 g cm^{-3} and shear viscosity 12.97 poise. The temperature for all the experiments was 29.5°C . Drops of carbon tetrachloride were injected by pouring from the top surface. Drops of silicone oil and air bubbles were formed under pressure at an orifice at the base of the column. The properties of each of the three systems are given in Table 1. No special precautions were taken to eliminate surface-active contaminants

TABLE 1 Physical properties of the systems

Dispersed fluid	Density of dispersed phase ρ_d (g cm ⁻³)	Viscosity of dispersed phase μ_d (poise)	Interfacial tension σ (dyne cm ⁻¹)	Approximate range of sphere-equivalent diameters over which pointed tails have been observed (cm)
Carbon tetrachloride	1.585	0.0105	34.3	1.13-1.70
Silicone oil	0.92	0.545	53.5	0.68-1.19
Air	0.00126	0.00018	73.0	0.58-0.98

from the liquids studied. In order to characterise the rheological behaviour of the aqueous sugar solution, an extensive series of tests was done with a rheometric mechanical spectrometer and with a Weissenberg Rheogoniometer (model RD-18). No dependence of viscosity on either rate of strain or on time could be observed so long as precautions were taken to ensure constancy of temperature and moisture content. Thus the continuous fluid can be taken to be Newtonian. The dispersed fluids (see Table 1) were also Newtonian.

Tracings of drops and bubbles with a teardrop shape, taken from still photographs, are presented in Fig. 1. Tails are observed only for a limited range of drop and bubble sizes as shown in Table 1. As the drop or bubble size is increased beyond the teardrop range, the tail disappears and is replaced by an indentation or dimple at the rear.

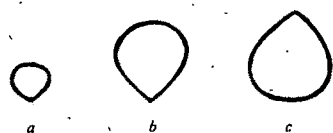


FIG. 1 Shapes of air bubbles, and drops of silicone oil and carbon tetrachloride moving through 70%, w/w, aqueous sucrose solution. a, air bubble; sphere equivalent diameter: 0.73 cm; velocity 3.08 s⁻¹. b, Silicone oil; 1.19 cm; 2.82 cm s⁻¹. c, Carbon tetrachloride; 1.56 cm; 1.80 cm s⁻¹.

Drops or bubbles which were given a teardrop shape by the injection process (for example, if the tail was stretched as a drop pulled away from the injection orifice) or by coalescence tended to retain the teardrop shape whereas those which began as spheres tended to remain so unless they coalesced with other drops or bubbles. In other words, a tail once formed was not retracted but spherical fluid particles in free rise or fall did not tend to form tails on their own. The tails observed were generally less pronounced than those reported by other workers for non-Newtonian liquids. Recently, tailing of a filament of the dispersed liquid has been observed for cases where the continuous fluid is significantly viscoelastic¹¹. No such phenomenon has been observed with the Newtonian liquids used in the present work.

We conclude that teardrop shapes are not restricted to non-Newtonian fluids as has been generally believed. On the other hand, the range over which pointed tails can be observed in Newtonian liquids seems to be small and there is little doubt that thixotropic and viscoelastic continuous liquids lead to more pronounced tails and to phenomena, such as trailing filaments, which do not occur for Newtonian liquids.

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Colonisation of the early ocean floor

THE earliest well documented evidence of macroscopic life is provided by stromatolitic algae from sediments deposited in intertidal or shallow marine environments at least 2,700 m.y. ago¹. It is generally agreed that the earliest metazoan animals were soft-bodied forms which are rarely preserved but have left tracks, trails and burrows, collectively known as trace fossils. The earliest reliably identified trace fossil is a burrow system considered to have been produced by a worm-like organism, probably an annelid, found in shallow marine clastic sediments of the Grand Canyon Series (USA) suggested to be over 1,000 m.y. old². Vertical burrows have been found in the Buckingham Sandstone in North Australia, also a shallow water deposit dated radiometrically as more than 790 m.y. old², and in the Areyonga Formation, of similar age³. A more extensive collection of trace fossils has recently been recorded by Webby⁴ from the clastic shallow marine late Precambrian (about 600 m.y. old) Torrawangee Group of New South Wales (Australia) with three trace fossil genera positively identified: *Planolites*, *Cochlichnus* and *Torrawanga*. The late Precambrian Ediacara Beds of South Australia, also interpreted as shallow water clastic deposits, have yielded one positively identified trace fossil genus (*Cochlichnus*) and five other forms, all thought to have been produced by metazoa². The common factor in these occurrences is the shallow water environment. Most of the trace fossils are burrows of infaunal deposit feeders which inhabited offshore environments of relatively quiet water; but the record of vertical burrows, now most commonly found in the high energy intertidal zone, may indicate occupation of even the shallowest water niches. There is as yet no indication of colonisation of the deep oceans during Precambrian times.

Tracing the dispersal of even soft-bodied benthonic animals from their initial shallow water habitat into deep water is helped by the facies dependence of the trace fossils which they produce. Seilacher⁵ suggested that many trace fossil genera can be assigned to one of three depth-controlled communities and this has, in general, been confirmed by later studies⁶⁻⁸. The communities, each named after a characteristic trace fossil are, in order of increasing water depth: *Cruziana* (littoral to wave base), *Zoophycos* (wave base to

TABLE 1 First appearance of ichnogenera

	<i>Cruziana/Zoophycos</i> community (shallow)	<i>Nereites</i> community (deep)	Ichnogenera independent of depth
Tertiary	0	5	0
Cretaceous	0	18	0
Jurassic	1	0	0
Triassic	2	0	1
Permian	2	1	0
Carboniferous	2	1	2
Devonian	0	2	0
Silurian	0	2	0
Ordovician	5	7	7
Cambrian	21	3	5
Precambrian	3	0	1

zone of turbidite deposition) and *Nereites* (deep water turbidite zone). Thus, by determining the oldest known occurrence of trace fossil genera belonging to the different communities, it should be possible to trace, in time, the dispersal of benthonic faunas into deep water, away from the shallow water niche which they occupied in the Precambrian. Also, by considering the stratigraphical range of the depth-controlled trace fossils, it should be possible to estimate the diversity of trace fossils, and hence animals, in each depth zone at different times. For this analysis, a wide, and as far as possible non-selective, coverage is necessary.

I have therefore considered all ichnogenera (except trace fossils produced by boring organisms) mentioned in a recently published comprehensive text on trace fossils⁹. This text includes papers on all systems and from widespread areas, so that all common traces and some rarer ichnogenera were included but old, inadequately described, ichnogenera were avoided. Each of the depth-dependent ichnogenera was then assigned to its community and its range noted from the *Treatise on Invertebrate Paleontology*¹⁰, modified where necessary to take account of more recent investigations. In all 81 ichnogenera were included.

The results are presented in Tables 1 and 2. Table 1 records only the first appearance of ichnogenera in the stratigraphic column. Table 2 is based on the stratigraphic range of ichnogenera and each ichnogenus is counted as present in every system within its stratigraphic range whether or not it has been recorded from them all.

The tables show a clear trend of increasing diversity in deep water, and possibly decreasing diversity in shallow water, with time. Extension of the survey to include all trace fossil genera in the *Treatise of Invertebrate Paleontology* made no significant difference to the trends but the results are not reported here because of the uncertainty in assigning many neglected and poorly described genera to a community. The diversification seen in the younger Precambrian rocks is continued and markedly accelerated in Lower Cambrian times. Abundant and surprisingly diverse

trace fossils have been recorded from shallow water Lower Cambrian sediments in many parts of the world^{2,11-16} but few forms have been found in deep water deposits. Even simple depth-independent forms are rare in this environment. The contrast between shallow and deep water in the numbers and diversity of trace fossils recorded persists throughout Cambrian times. It cannot be due to any lack of suitable deep water deposits because these are widely distributed and the contrast is clearly seen, for example, in Wales where, despite the great number and variety of traces found in the shallow water sediments, extensive searching in the well exposed deep water turbidite sequences has yielded only a few mostly non-diagnostic burrows¹⁷. Significant colonisation of the deep ocean floor does not therefore seem to have taken place before the close of the Cambrian period.

The widespread shallow water seas of earliest Ordovician (Arenig) times supported an abundant and varied benthos which produced many trace fossils, particularly trilobite traces and infaunal burrows. The first traces assignable to the *Zoophycos* community appeared, including *Zoophycos* itself, which has been recorded low in the Ordovician sequence in Iraq⁵, thus testifying to the colonisation of the intermediate depths. There is also evidence of the first significant colonisation of the deep ocean floor with nine ichnogenera recorded from the *Nereites* community. There is, in addition, an increase in the number of depth-independent ichnogenera from 6 to 13. Data from Upper Palaeozoic and Mesozoic strata suggest the possibility of a further decrease in the number of shallow water forms. The well known abundance of trace fossils in the late Mesozoic and Tertiary flysch successions¹⁸⁻²³ confirms an extensive colonisation of deep water at this late stage, although the very sharp increase in number of ichnogenera present may partly reflect the more detailed study to which these deposits have been subjected. Thus, from the Cambrian to the Tertiary, the available data suggest that the total number of ichnogenera has remained roughly constant, but there has been an increase in the diversity in deep water and possibly a decrease in diversity in shallow water.

The initial colonisation of the deep oceans may have been stimulated by high dispersal pressures in the shallow water niches. Trace fossil abundance and diversity suggest, however, that such pressures reached a high level as early as Lower Cambrian times, yet significant colonisation of the deep oceans was apparently delayed by some 70 m.y., until the Ordovician, when dispersal pressures do not appear to have been much greater. This delay may have been the result of low oxygen concentration in the early deep seas. Most deep water benthos are, however, sediment grazing types; their inability to colonise this environment during the Cambrian may also reflect inadequate supplies of organic detritus within the muds of the early deep ocean floor, together with anaerobic decay brought on by oxygen deficiency in such organic material as may have been present.

No doubt the stratigraphical range of many of the trace fossils used in this analysis will be extended as a result of further work. Nevertheless, the main trends are so clearly shown that it is unlikely that such additions will significantly modify the conclusions which are:

(1) The earliest benthonic metazoa shared a shallow water niche with already existing stromatolitic algae but the algae were restricted to calcareous facies while metazoan trace fossils have so far only been found in clastic sediments.

(2) There was rapid diversification in tracks, trails and burrows in early Cambrian shallow water environments. In contrast, only three genera of deep water trace fossils have so far been recorded from Cambrian sediments and significant colonisation of the deep ocean floor appears to have been delayed by some 70 m.y. until the Ordovician when at least nine ichnogenera were present.

TABLE 2 Number of ichnogenera present during different periods

	<i>Cruziana/Zoophycos</i> community (shallow)	<i>Nereites</i> community (deep)	Ichnogenera independent of depth	Total
Tertiary	12	30	9	51
Cretaceous	18	31	11	60
Jurassic	19	12	13	44
Triassic	19	12	13	44
Permian	18	12	12	42
Carboniferous	22	13	14	49
Devonian	21	12	12	45
Silurian	22	9	12	43
Ordovician	24	9	13	46
Cambrian	24	3	6	33
Precambrian	3	0	1	4

(3) In the late Mesozoic and early Tertiary there appears to have been a sharp increase in abundance and variety of trace fossils produced in deep water but this may partially reflect the more detailed study to which these deposits have been subjected.

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BIOLOGICAL SCIENCES

mRNA is expected to form stable secondary structures

Most sequenced RNAs have the potential to form secondary structures by means of local base pairing and loop formation. Arguments have been advanced that these structures are the result of evolutionary pressure, or of requirements for unique functions such as virus packaging¹⁻⁷. To determine whether secondary structure is an intrinsic or anomalous property of RNA we have investigated random sequences generated by computer. These sequences, which contain no real biological information, can be arranged into thermodynamically stable secondary structures with about 50% base pairing. The results imply that one should expect a high degree of base pairing in RNA, consistent with early estimates of helical content in random copolynucleotides⁸

TABLE 1 Pairing percentages in random structures

Sequence length	No. investigated	% of bases paired
37-62	23	42
77	25	55
144	2	52

and the constraints accompanying structure formation in a hypothetical tRNA molecule^{9,10}. Therefore, the mere existence of secondary structure does not necessarily implicate special biological functions, although the potential for functional interactions clearly exists.

Table 1 shows the average percentage of bases paired in fifty random sequences of various lengths. A sequence was generated by assigning equal *a priori* probabilities to each base and using a standard random number routine to decide which base occupied a particular position along the chain. In each case the computer printed out the base pairing matrix¹¹, facilitating the choice of pairing arrangements by inspection. These arrangements were then evaluated according to rules detailing thermodynamic stability¹¹⁻¹⁵, and the most stable secondary structure was selected in each of the fifty cases. It is possible that occasionally even more stable structures were overlooked; this implies that the resulting base pairing percentages (Table 1) are minimum values. Nevertheless, the most stable structures on the average have 40-60% of bases paired (an example of a random sequence with about 60% pairing is shown in Fig. 1).

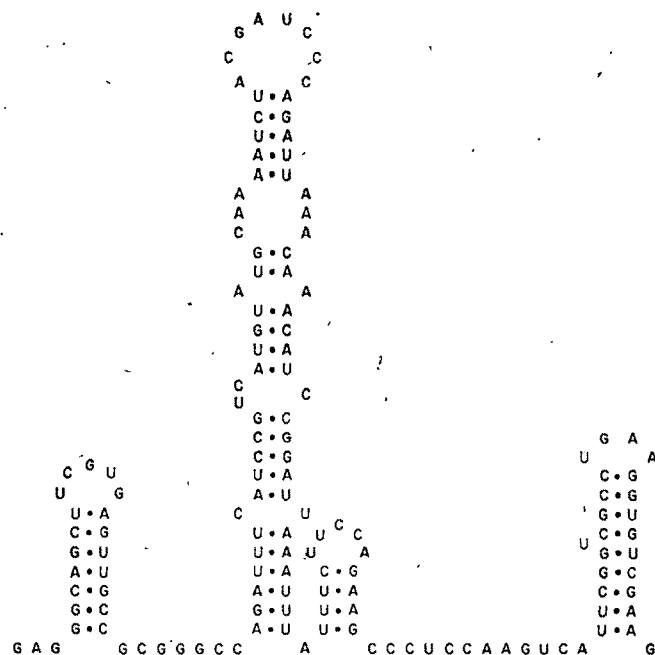


Fig. 1 One possible secondary structure for a random sequence of 140 nucleotides. Approximately 60% of the bases are paired.

At fixed length the extent of base pairing observed is not highly variable. Fig. 2 shows the distribution of base pairing in twenty-five random sequences of seventy-seven nucleotides each. Fully 80% of the most stable structures contain 21 ± 3 base pairs, and no structure includes fewer than 15 or more than 28 base pairs. Even though the number of sequences evaluated is small, statistical analysis¹⁶ indicates that these results are applicable to sequences in general with a high level of confidence. The probability that the mean of the distribution shown in Fig. 2 is within $\pm n$ base pairs of the mean of an infinitely large sample is 0.92 for $n =$

1 and 0.99 for $n = 2$. Therefore, the probability of finding a 77-nucleotide RNA, random or real, without secondary structure is very small.

Transfer RNAs (average length of 77 bases) include on average 21 base pairs, but the universality of the resulting clover leaf secondary structure implicates evolutionary selection processes. If tertiary structure and base modifications are ignored, about half the natural sequences can be arranged into structures more stable than the clover leaf. (Many of these retain some elements of the clover leaf, usually the anticodon and acceptor stem. However, the existence of an acceptor stem-like feature in our random structures is an improbable event). The glycyl tRNA from *E. Coli* (Fig. 3) is the most striking example we encountered. It seems possible that selected base modification and specific tertiary interactions prevent these structures from forming under physiological conditions. However, experiments in non-physiological buffers should be interpreted with great care since at low ionic strength extended structures (for example, Fig. 3) may form which minimise the electrostatic free energy.

This structural polymorphism emphasises the need for accurate evaluation of the stability of proposed structures. Optimisation of base pairing is not the only determinant of stability since the size and number of loops is also important. Large internal loops, for example, involve considerable free energy cost and are often overlooked in stability calculations. (Examples include the acceptor stem in tRNA¹⁷ and the flower model 'stalk' in the MS II coat protein gene¹⁸.) When alternative structures are possible that with lowest free energy should be selected. Confirmation is sometimes possible by physical¹⁹ or ribonuclease-digestion techniques¹⁸.

The relationship between the presumably conflicting demands of mRNA secondary structure and amino acid sequence has been the subject of much discussion²⁰⁻²². Our results do not support the hypothesis that amino acid sequences have evolved in part due to pressure for maintaining stable RNA base pairing²². Sequences generated at random exhibit pairing percentages only marginally less than those of the MSII coat protein gene. The distribution of base pairs

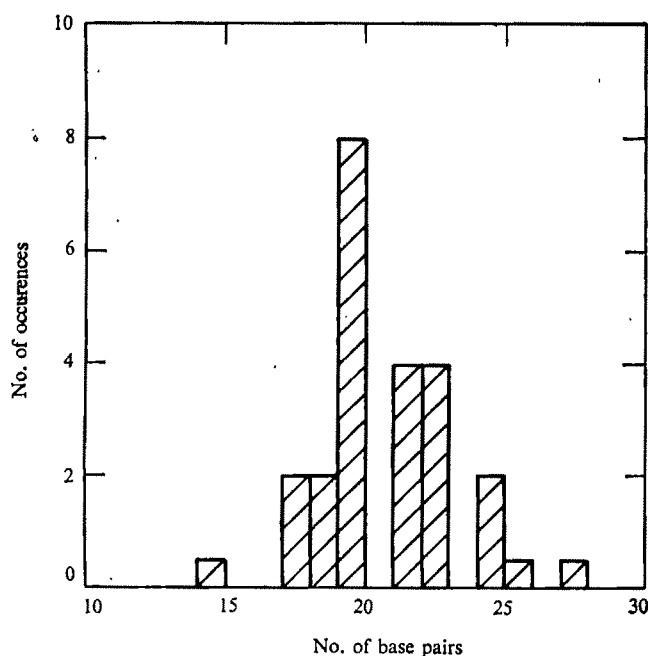


Fig. 2 A base pair distribution obtained by randomly generating twenty-five chains, each with seventy-seven base pairs. The height of the shaded area indicates the number of structures which contains the given number of base pairs. The distribution has a mean of 21.3 base pairs and a standard deviation of 2.84.

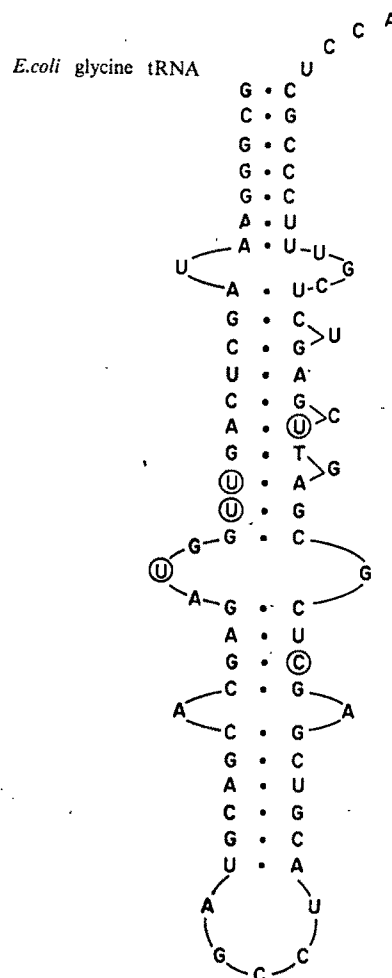


Fig. 3 A possible secondary structure from *E. coli* glycyl tRNA. The circled bases are modified and do not pair in the clover leaf structure.

in this gene involving degenerate codons²² is consistent with evolutionary pressure on the RNA sequence, or pressure on the protein amino acid sequence, or no pressure at all within statistical uncertainty. Actually, there is no obvious need for RNA to evolve toward structure of minimum free energy. Rather, specific structures should evolve for specific purposes. Defects in pairing which result in instability might even be useful in recognition functions; at the very least they would conserve energy required to disrupt base pairing during translation.

The extent of base pairing exhibited by random structures implies that all mRNAs will form secondary structures. Such high percentages arise naturally from sequences composed of four different types of bases with three allowed pairing possibilities. The absence of secondary structure in mRNA would be surprising and significant. One such example is the polyadenylate sequences present in HnRNA. There is no apparent need to postulate unusual functions or evolutionary pressure on amino acid sequence to explain the existence of stable base pairing in RNA. The results, however, do not imply that there is no evolutionary pressure on RNA sequences, but rather suggest that the existence of such pressure cannot be readily deduced from the observation that a particular messenger has 60% of its bases paired. Finally, it has been suggested that single stranded DNA may be important for control of chromosomal functions²³. Should extensive single stranded regions of average composition exist *in vivo*, they would likely have the potential for formation of stable secondary structure.

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Hepatoma—foetal Phe-tRNA also present in normal rat liver

SEVERAL groups have demonstrated independently, the presence of two isoaccepting species of Phe-tRNA in rat hepatomas and only one in normal rat liver. By use of reversed phase chromatography on RPC-2 columns¹ two groups²⁻⁴ demonstrated an early eluting, minor peak of Phe-tRNA in Morris hepatomas 5123C and 5123D, which was not seen in normal rat liver tRNA, while one group^{5,6} reported the same extra peak in hepatoma 9618A but not in 3924A. We too have observed this minor peak of Phe-tRNA in Morris hepatomas 5123D, 7777 and 7800 using RPC-2 chromatography. Figure 1a shows a typical RPC-2 pattern for these three hepatomas all of which had a minor Phe-tRNA peak containing 14–15% of the total acid-precipitable counts. Recently Gonano *et al.* reported a similar peak of Phe-tRNA in foetal rat liver which cochromatographs on RPC-2 with the minor peak from hepatoma 5123C⁷.

Recent work in this laboratory, comparing aminoacyl-tRNAs from mouse and rat tumours, has used another reversed phase column system, RPC-5⁸, and fresh isolations of tRNA and aminoacylating enzymes from tumours and normal tissues. The RPC-5 profiles were basically in agreement with those from RPC-2 runs of older preparations and

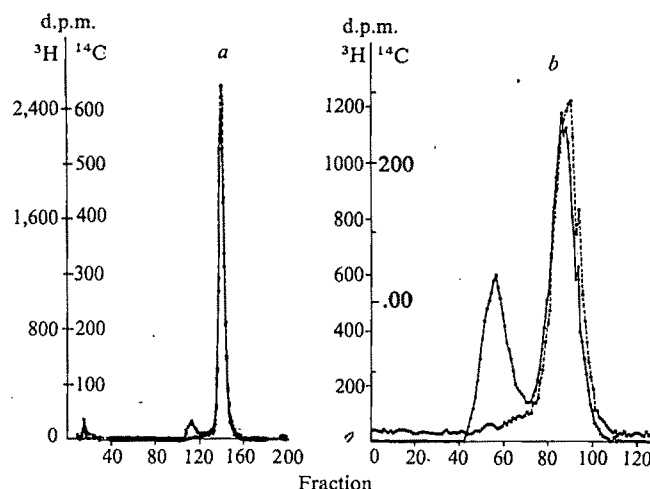


Fig. 1 a, Elution pattern from an RPC-2 column⁴ on which ³H-Phe-tRNA from Morris hepatoma 7800 (—○—) was cochromatographed with ¹⁴C-Phe-tRNA from normal adult Buffalo rat liver (—●—). b, Elution pattern from an RPC-5 column⁸ on which ³H-Phe-tRNA from normal adult Buffalo rat liver (—○—) was cochromatographed with ¹⁴C-Phe-tRNA from Morris hepatoma 7777 (—●—). Phe-tRNAs were prepared by incubating Sephadex G-100-purified¹³ 4S RNA with aminoacylating enzymes (see below) in a reaction mixture containing 5mM Na₂ATP (neutralised), 100 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 14 mM β-mercaptoethanol, 0.25 mM each of nineteen amino acids (minus phenylalanine) and 4 × 10⁻⁵–7 × 10⁻⁵ M ³H-L-phenylalanine or ¹⁴C-L-phenylalanine for 30 min at 37° C. The aminoacylating enzymes were prepared from an homogenate of liver or tumour in medium A1 (0.25 M sucrose, 0.006 M MgCl₂, 0.015 M Tris-HCl, pH 7.6, 0.8M KCl and 0.014 M β-mercaptoethanol) from which the cellular membranes and ribosomes had been removed by centrifugation at 100,000g for 2 h. This was absorbed to and eluted from a column of DEAE cellulose by the method of Muench and Berg⁹ and stored in 50% glycerol at -20° C for months (DEAE enzyme), or the S-100 was merely passed over a small column of Sephadex G-10 equilibrated with medium A1 minus sucrose and stored in 50% glycerol at -20° C (G-10 enzyme). After three phenol extracts the labelled Phe-tRNAs were precipitated with ethanol at -20° C, dissolved in column buffer, desalted on Sephadex G-25, mixed and applied to the column specified. The RPC-2 column (1 cm × 240 cm) was eluted at 15° C with a 2 l linear gradient from 0.35 M to 0.65 M NaCl in 0.01 M Na acetate and 0.01 M Mg acetate, pH 4.5. Fractions (10 ml) were collected and precipitated with 1 ml 50% trichloroacetic acid at 4° C, and the precipitates were collected on Millipore filters which were dried, treated with 0.2 ml 1 M KOH for 5 min at room temperature, neutralised with 1 M HCl and counted in a liquid scintillation counter. Three replicate counts were averaged and converted to absolute activity on an IBM 1620 computer. The RPC-5 column (0.5 cm × 50 cm) was eluted at 22° C with a 400 ml linear gradient from 0.5 M to 0.65 M NaCl in 0.01 M Na acetate, 0.01 M Mg acetate and 0.001 M β-mercaptoethanol. Fractions (2.5 ml) were collected, precipitated with 2.5 ml 10% trichloroacetic acid at 4° C, and processed as above.

showed an early eluting peak of Phe-tRNA in hepatomas but not in normal liver (Fig. 1b). While conditions for optimal aminoacylation of phenylalanine to each batch of tRNA were being retested, the DEAE cellulose preparation of aminoacylating enzymes (DEAE enzymes)⁹ from liver or tumour was found not to be as effective in the extent of aminoacylation as was a cruder preparation made by passing a 100,000g supernatant of tissue homogenate over a small column of Sephadex G-10 (G-10 enzymes). The optimal aminoacylating conditions were the same for enzymes prepared by either procedure. RPC-5 chromatography of tRNA from all three hepatomas aminoacylated with G-10 enzymes showed a more prominent early eluting peak of Phe-tRNA (Fig. 1b) than that found by RPC-2 chromatography of tRNA aminoacylated with DEAE enzyme (Fig. 1a). Further, liver tRNA aminoacylated using the G-10 enzyme showed

the presence of a prominent minor peak of Phe-tRNA which had not been seen when the DEAE enzyme preparation was used (Fig. 2).

Because a loss of activity might have occurred during storage of the DEAE enzyme preparation at -20°C in 50% glycerol, a fresh preparation was made from normal rat liver by the procedure used before. Indeed the fresh enzyme was more active in aminoacylating phenylalanine to liver tRNA but not to the extent of a fresh or stored G-10 enzyme preparation. The fresh DEAE enzyme preparation also revealed a very small minor Phe-tRNA peak (Fig. 2), but this was always smaller than the minor peak revealed when the same liver tRNA was aminoacylated with G-10 enzyme.

The G-10 enzyme preparation contained some endogenous tRNA, but the earlier eluting peak of Phe-tRNA was not

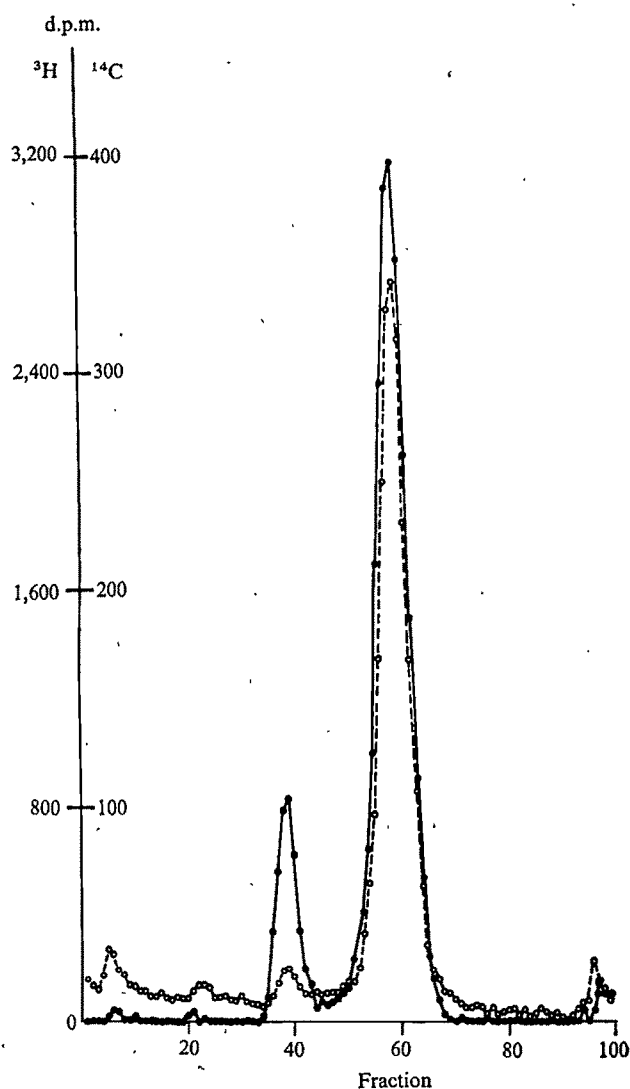


Fig. 2 Elution pattern from an RPC-5 column showing the effect of aminoacylating enzymes prepared by two methods on the pattern of Phe-tRNA prepared from normal rat liver. tRNA was aminoacylated with ^3H -L-phenylalanine using liver enzymes freshly eluted from DEAE cellulose (stored at 20°C for less than 7 d) (---○---) and mixed with tRNA from the same source which had been aminoacylated with ^{14}C -L-phenylalanine using liver cytosol merely passed over Sephadex G-10 (—●—) (see Fig. 1 legend for conditions of enzyme preparations and the aminoacylation reaction). The RPC-5 column (0.5 cm \times 30 cm) was developed under high pressure¹⁴ at 22°C with a 150 ml gradient of NaCl from 0.5 M to 0.8 M in 0.01 M Na acetate, 0.01 M Mg acetate and 0.001 β -mercaptoethanol. Fractions (1.2 ml) were collected directly in glass vials and counted in a liquid scintillation counter. Two replicate counts were averaged, and absolute activity was calculated in a PDP-11 computer.

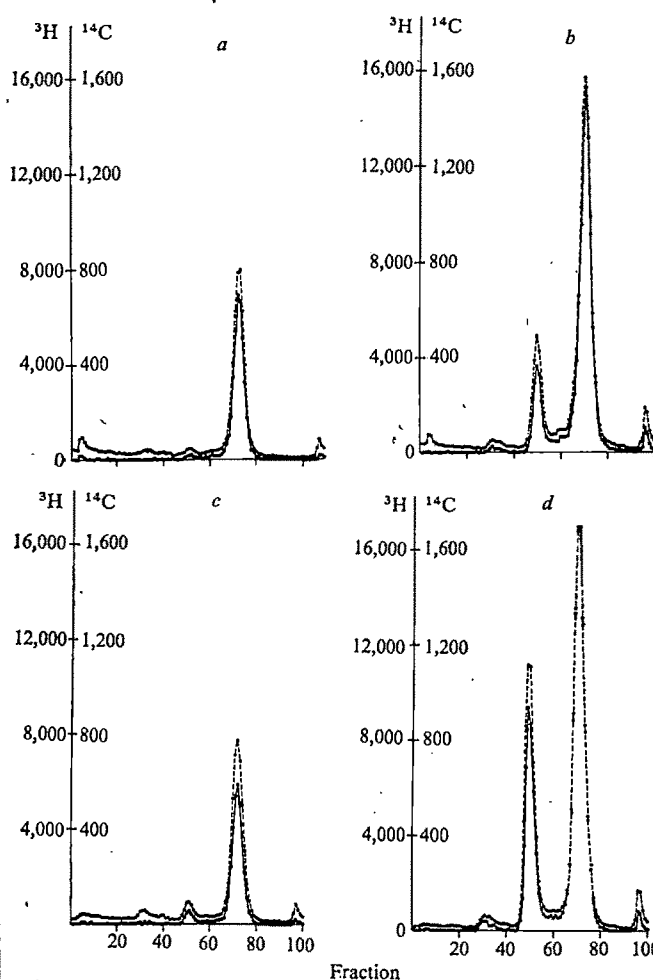


Fig. 3 Four elution patterns from RPC-5 columns using conditions indicated in the legend for Fig. 2. tRNA (30 μg) from normal rat liver (a and b) or Morris hepatoma 5123D (c and d) were aminoacylated with ^3H -L-phenylalanine and chromatographed with 45 μg tRNA from the source that had been aminoacylated with ^{14}C -L-phenylalanine. Reaction conditions are given in the legend for Fig. 1, and the type and source of enzyme used was as follows. a: ---○---, ^3H -Phe, liver RNA, liver DEAE enzyme; —●—, ^{14}C -Phe, liver RNA, 5123 DEAE enzyme. b: ---○---, ^3H -Phe, liver RNA, 5123 G-10 enzyme; —●—, ^{14}C -Phe, liver RNA, liver G-10 enzyme. c: ---○---, ^3H -Phe, 5123 RNA, liver DEAE enzyme; —●—, ^{14}C -Phe, 5123 RNA, 5123 DEAE enzyme. d: ---○---, ^3H -Phe, 5123 RNA, 5123 G-10 enzyme; —●—, ^{14}C -Phe, 5123 RNA, liver G-10 enzyme.

found in it. Mixtures of DEAE and G-10 enzyme preparations yielded Phe-tRNA identical in amount and pattern to that produced by G-10 enzyme only, ruling out the presence of an inhibitor in the DEAE enzyme.

The effect of the two enzyme preparations on tumour tRNA was then studied. Morris hepatoma 5123D and normal rat liver were used as the sources of tRNA and enzymes. Identical amounts of liver tRNA (Fig. 3a and b) and tumour tRNA (Fig. 3, c and d) were aminoacylated with DEAE and G-10 enzymes from liver and tumour in all four combinations and plotted on the same scale. The same type of enzyme preparation, whether from normal or neoplastic tissue, gave the same extent and pattern of aminoacylation of phenylalanine to a particular tRNA preparation. G-10 enzymes increased the total incorporation into the major peak of Phe-tRNA two to three-fold over DEAE enzymes while increasing the incorporation into the minor peak more than fifteen-fold, such that a minor peak not seen in liver tRNA charged with aged DEAE enzyme became very prominent after G-10 enzyme had been used. Furthermore, there

was a distinct difference in the prominence of the minor Phe-tRNA from normal liver and from hepatomas no matter which aminoacylating enzyme was used, but it no longer seems to be accurate to refer to a tumour-specific peak of Phe-tRNA or one completely absent in normal rat liver. In fact, the presence of the minor peak of Phe-tRNA in normal liver has been reported by Tidwell *et al.*¹⁰ in a detailed study of normal and regenerating rat liver.

Possibly a more interesting aspect of these findings is the difference between the two enzyme preparations that enables the minor peak of normal Phe-tRNA to be demonstrated. Work is underway to investigate several possibilities, for example, that DEAE cellulose purification removes or inactivates one of several Phe-tRNA synthetases or a tRNA-modifying enzyme. Tumour tissue may contain such an excess of this factor that the DEAE cellulose step does not remove all its activity.

These investigations have not yet included a study of foetal tRNA, but we suggest that the striking abundance of the early-eluting Phe-tRNA peak in foetal rat liver, reported by Gonano *et al.*⁷, may also be associated with a great abundance of that activity we find deficient in our DEAE enzyme but abundant in the G-10 preparations. The association of neoplasia and foetal gene activation is particularly provocative and this increased amount of a normally minor Phe-tRNA in foetal and neoplastic hepatic tissue should be explored further, remembering that equally significant changes may be occurring simultaneously in the enzymes involved in tRNA aminoacylation. Another product of foetal liver, α -foetoprotein, has also been associated with hepatomas in several species. Apparently α -foetoprotein is secreted by only a limited number of rat hepatomas¹¹, with abnormally high serum levels reported for Morris hepatomas 3924A and 7777 but not for 9618A, 5123D or 7800, all of which (except 3924A⁸) have a prominent peak of the minor Phe-tRNA. Experiments with regenerating rat liver also suggest a transient increase in α -foetoprotein secretion¹², but only normal amounts of the minor Phe-tRNA peak have been reported 12, 18 and 24 h after partial hepatectomy¹⁰. We have not found increases in the minor Phe-tRNA in regenerating liver taken 1, 2, 3, 7, and 10 d after partial hepatectomy. So although both the minor Phe-tRNA and α -foetoprotein may represent increased activation of genes primarily associated with embryogenesis or rapid growth conditions, a direct correlation does not exist between them.

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Activity of DNA templates during cell division and cell differentiation

THE rates of cellular RNA and DNA synthesis decline during the mitotic stages of cell division¹ and during cell differentiation². The molecular mechanisms mediating such a decline may include changes in the size of mononucleotide pools, changes in RNA and DNA polymerase activity, or changes in DNA template activity³, but previous studies have not distinguished among these possibilities. We have developed a high-resolution electron microscopic technique for directly analysing DNA template activity within living cells⁴, and now report its application to individual cells undergoing division and differentiation in human bone marrow.

Bone marrow spicules⁵ and peripheral blood cells⁶ were isolated under sterile conditions from patients as previously described, and were subjected to the analysis of active DNA templates while in the living state⁴. Acridine orange was used as previously described⁴ to probe for the location and number of active DNA templates within each cell⁵. The location of each probe site was determined by electron microscopic visualisation of the electron-dense reaction product formed between acridine orange and osmic acid⁵. Control experiments omitting either the acridine orange or the DNase steps from the reaction sequence did not reveal any reaction product⁵. Positive cells revealed large (>0.1 μ m) or small (0.025–0.1 μ m) reaction products localised exclusively within the nuclear euchromatin complexes⁵. These probe sites were counted within each cell by one observer, and another observer independently classified the stage of cell division or of cell differentiation, using the ultrastructural criteria previously reported for dividing cells⁷, differentiating granulocytes⁸, and differentiating erythrocytes⁹.

A total of fifty dividing normal bone marrow cells were analysed (Table 1). These analyses revealed a progressive decrease in the number of probe sites per cell through the early and late stages of prophase, to an absence of any probe sites in metaphase, anaphase and early telophase, with a subsequent rapid increase in the number of probe sites per cell in late telophase, returning to a basal level in interphase.

Similarly a total of 123 normal differentiating granulocyte precursor cells and 176 normal differentiating erythrocyte precursor cells (Table 2) were analysed. These analyses revealed a progressive decrease during the course of normal cell differentiation in the percentage of cells containing either large or small probe sites. In addition, these analyses revealed a progressive decrease during cell differentiation in the number of large probe sites per positive cell, as well as a progressive decrease in the fraction of all probe sites represented

TABLE 1. Probe counts in dividing bone marrow cells

Stage of cell division	Mean probe count per cell
Early prophase	35.3
Late prophase	14.8
Metaphase, anaphase, early telophase	0
Late telophase	85.0
Interphase	35.1

TABLE 2 Probe counts within differentiating marrow cells

Granulocytes Stage of cell differentiation	% Cells containing		Mean probe count per positive cell	
	Large probes	Small probes	Large probes	(Large probes)/ (total probes)
Promyelocytes	91.5	100.	9.55	0.19
Myelocytes	85.9	96.4	8.75	0.18
Metamyelocytes	47.6	85.7	3.9	0.05
Band granulocytes	0	20.0	0	0.0
Segmented granulocytes	0	12.2	0	0.0
Erythrocytes Proerythroblasts	100	100	16.0	0.65
Early erythroblasts	84.7	91.6	8.5	0.32
Late erythroblasts	14.8	17.2	3.4	0.20
Nucleated erythrocytes	0	8.3	0	0

by large probe sites (Table 2). These observed decreases in DNA template activity during the course of normal bone marrow cell differentiation are statistically highly significant ($P < 0.01$) by Mann's test for trend significance¹⁰.

Independent analyses have previously revealed a direct correlation between DNA template activity as measured by probe sites within isolated chromatin, and the rate of RNA synthesis by such isolated chromatin when supplied with excess amounts of exogenous RNA polymerase and monoribonucleotides¹¹. The studies reported here measuring probe sites within intact living cells similarly correlate closely with previous independent studies demonstrating a progressive decrease in RNA and DNA synthesis and cell division during normal bone marrow cell differentiation². These quantitative data suggest that a progressive restriction of DNA templates is an important mechanism during normal cell division and bone marrow cell differentiation¹². We are currently studying the molecular species¹³ mediating such restriction and release of DNA template activity during normal⁶ and leukaemic cell division and differentiation¹⁴.

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Functional instability of T7 early mRNA

It has been known for some time that mRNA of bacteriophage T7 is stable. Summers¹ measured the decay of acid-insoluble radioactivity in the mRNA after new synthesis was stopped with actinomycin in T7-infected, actinomycin-permeable *Escherichia coli* cells. The half-life of T7 mRNA was estimated greater than 25-30 min at 30° C compared with about 2.5 min for *E. coli* mRNA. Marrs and Yanofsky² showed that the mRNA transcribed from the tryptophan operon is degraded normally in T7-infected *E. coli* but T7 mRNA is stable, measured by hybridisation with T7 DNA. In these experiments only chemical stability of T7 mRNA was studied in terms of acid-insoluble amount or DNA-hybridisable amount and no information was obtained on the functional integrity of the mRNA.

During the late period of T7 infection, only late proteins are synthesised. If T7 early mRNAs are functionally stable and present in the cell together with late mRNA, preferential synthesis of late proteins must depend on some sort of translational control exerted at the level of protein synthesis. However, no discriminatory translational control against early mRNA in T7-infected cells has been detected (submitted for publication). Therefore, we have examined the functional and structural integrity of T7 early mRNA produced by an amber mutant, T7 am193. Since the amber mutation is in gene 1 coding for T7-specific RNA polymerase³, this mutant phage infection on a non-permissive host makes only early mRNA but no late mRNA.

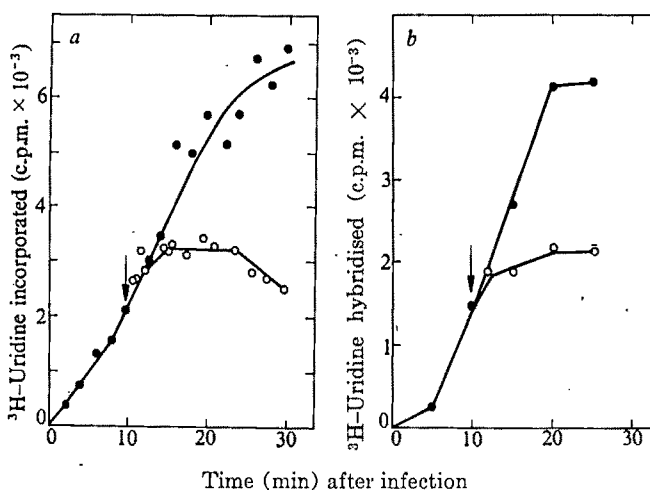


Fig. 1 RNA synthesis in ultraviolet-irradiated D10 F-cells infected with T7 am193. a, ³H-uridine incorporation into RNA; b, T7 DNA-hybridisable ³H-uridine radioactivity in RNA. a, *E. coli* D10 F- was grown in M9 medium at 30° C to a cell density of 6×10^8 ml⁻¹. The culture was chilled and transferred to a glass Petri dish, irradiated for 7 min with two Sylvania germicidal lamps G15TB at 25 cm, then shaken at 30° C for 5 min. Survival was less than 10^5 ml⁻¹. The irradiated culture was then infected with T7 am193 at a multiplicity of 10 and labelled with ³H-uridine (5 μ Ci in 3 μ g ml⁻¹). After 10 min at 30° C, the culture was divided into two. One portion was diluted with an equal volume of pre-warmed medium, the other with pre-warmed medium containing 800 μ g ml⁻¹ rifampicin. Samples of 0.025 ml before dilution and 0.05 ml after dilution were withdrawn and acid-insoluble radioactivity was counted. ●, ³H-uridine incorporation in T7 am193-infected cells; ○, ³H-uridine incorporation in T7 am193-infected cells treated with rifampicin at 10 min (arrow). b, Experimental conditions were the same as described in (a) except that 20 μ Ci in 1 μ g ml⁻¹ of ³H-uridine was added. Samples of 5 ml before and 10 ml after dilution were withdrawn and radioactive RNA was prepared from each. 1.6 μ g of RNA was hybridised to T7 DNA bound to filters¹². A mean value of duplicate samples was plotted. ●, Hybridisable ³H-count in RNA from T7 am193-infected cells; ○, hybridisable ³H-count in RNA from T7 am193-infected cells treated with rifampicin at 10 min (arrow).

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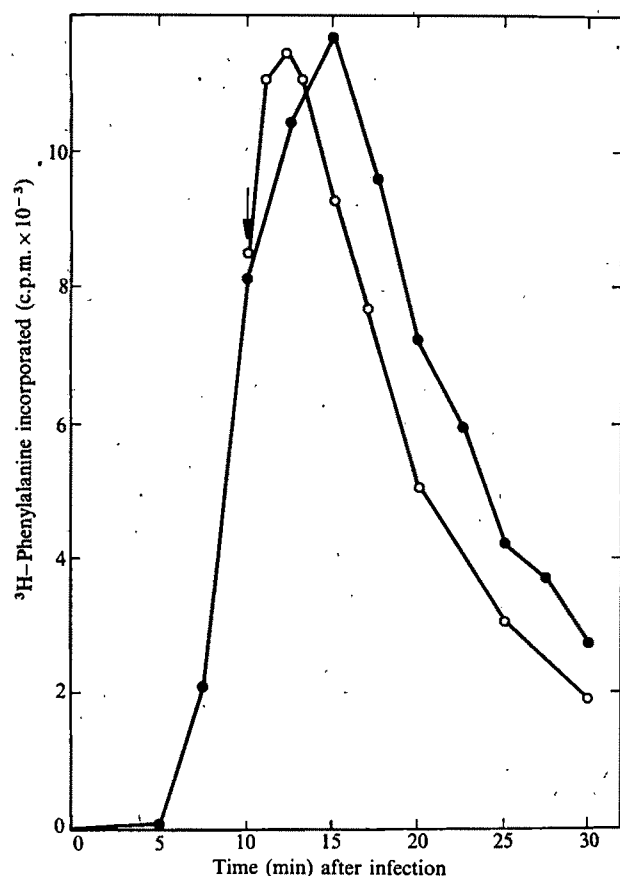


Fig. 2 Functional instability of T7 *am193* mRNA in directing *in vitro* protein synthesis. Ultraviolet-irradiated *E. coli* D10 F⁻ was infected with T7 *am193* as described in the Fig. 1 legend. RNA was extracted from the samples withdrawn at indicated times and the messenger activity of the RNA to direct *in vitro* protein synthesis was tested. The cell-free protein-synthesising system contained 5 ²⁶⁰A units of IS₆₀ (pre-incubated cell-free extract) from uninfected D10 cells and 5 μ Ci ³H-phenylalanine in a total volume of 0.25 ml. RNA (40 μ g) from each sample was added as messenger. Incubation was at 35° C for 30 min and hot acid-precipitable radioactivity was counted. ●, Messenger activity of RNA from T7 *am193*-infected cells; ○, messenger activity of RNA from T7 *am193*-infected cells treated with rifampicin at 10 min (arrow).

Our results described here confirmed the chemical stability of T7 early mRNA. We found, however, that functional activity of T7 early mRNA, tested in an *in vitro* protein-synthesising system, decayed at a rather rapid rate, a half-life about 6.5 min at 30° C. We believe that the functional decay is related to a loss of structural integrity of T7 early mRNA without losing much chemical quantity, because acrylamide-agarose gel electrophoresis revealed that the size of T7 early mRNA became smaller parallel to the loss of functional activity.

We used ultraviolet-irradiated bacteria in order to reduce host RNA synthesis after T7 infection. *E. coli* D10 F⁻, derived from D10 by acridine orange treatment (submitted for publication), was irradiated and then infected with T7 *am193*. In ultraviolet-irradiated cells, T7-directed synthesis of macromolecules proceeded somewhat slower than in normal cells. ³H-Uridine incorporation into RNA is shown in Fig. 1a. Ultraviolet-irradiated cells showed an accumulation of T7 early mRNA. Addition of rifampicin effectively shut off new initiation of RNA synthesis but the acid-insoluble radioactivity of the RNA declined only slightly after the addition of rifampicin.

Figure 1b shows the accumulation of T7 early mRNA which can be hybridised to T7 DNA. Ultraviolet-irradiated D10 F⁻ cells were infected with T7 *am193* and labelled with

³H-uridine. RNA was extracted from the cells taken at indicated times. A small amount of RNA synthesised by ultraviolet-irradiated cells without T7 infection did not have any hybridisable radioactivity (not shown in Fig. 1b). Confirming the result of Marrs and Yanofsky², we observed the stability of hybridisable T7 early mRNA even in the presence of rifampicin.

When T7 early mRNA was isolated from ultraviolet-irradiated, T7 *am193*-infected cells and tested for functional activity in an *in vitro* protein-synthesising system, we found that T7 early mRNA lost functional integrity at a rather rapid rate (Figure 2). RNA was extracted from aliquots of the infected culture at various times and added to a protein-synthesising system prepared from uninfected D10 F⁻ cells, and ³H-phenylalanine incorporation was measured. Protein synthesis-directing activity of the RNA decayed at a rapid rate after 15 min of infection even in the absence of rifampicin (Fig. 2). With rifampicin, functional decay of the RNA started earlier. The rate of decay with or without rifampicin was, however, similar.

Figure 3 shows f-met-tRNA binding to ribosomes directed by the RNA taken from ultraviolet-irradiated, T7 *am193*-infected cells. Salt-washed ribosomes and crude initiation factors were prepared from uninfected D10 F⁻ cells. Functional activity of T7 early mRNA in directing f-met-tRNA binding also decayed rapidly (compare with Fig. 3). Decay again started earlier in the presence than in the absence of rifampicin.

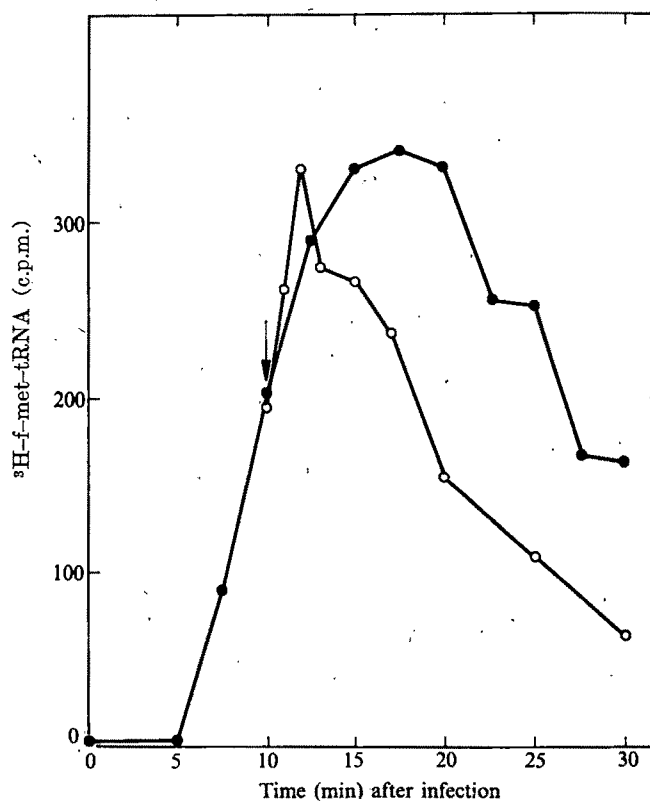


Fig. 3 Functional instability of T7 *am193* mRNA in directing the binding of f-met-tRNA to ribosomes. The same RNA samples from the experiment of Fig. 2 were used to direct initiation factor-dependent f-met-tRNA binding to ribosomes. The reaction mixture (submitted for publication) contained 75 μ g of 1 M NH₄Cl-washed ribosomes and 8 μ g unfractionated initiation factors from uninfected D10 F⁻ cells and ³H-f-met-tRNA (1,500 c.p.m. pmol⁻¹) in a total volume of 0.2 ml. RNA (54 μ g) from each sample was added as messenger. Incubation was at 35° C for 15 min and radioactivity of ³H-f-met-tRNA retainable on a Millipore filter was counted. ●, Messenger activity of RNA from T7 *am193*-infected cells; ○, messenger activity of RNA from T7 *am193*-infected cells treated with rifampicin at 10 min (arrow).

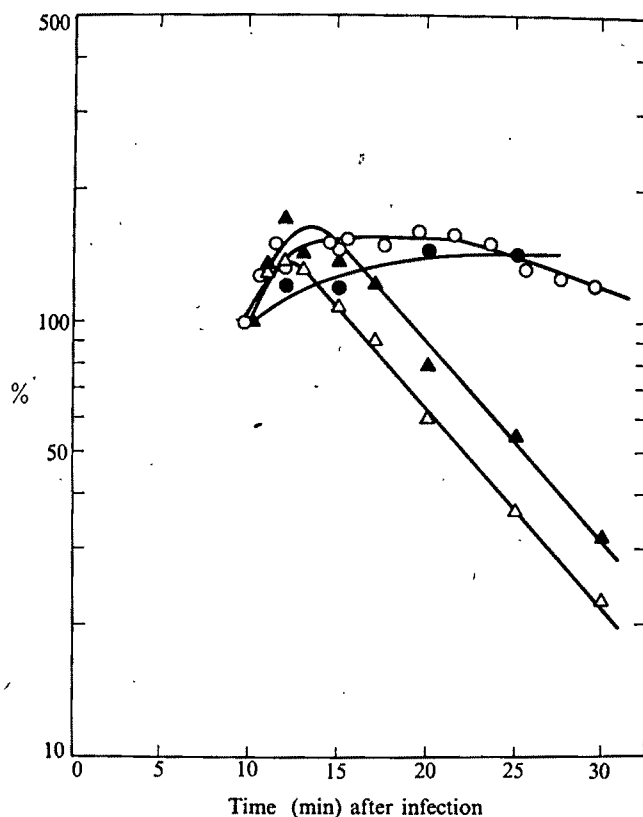


FIG. 4 Chemical stability and functional decay of T7 mRNA. Results of Figs 1, 2 and 3, obtained from the ultraviolet-irradiated cells infected with T7 *am193* and treated with rifampicin at 10 min, were replotted. The 100% value represents the value at the time of rifampicin addition (10 min after infection). ●, Acid-insoluble radioactivity in RNA (Fig. 1a); ○, T7 DNA-hybridisable radioactivity in RNA (Fig. 1b); △, messenger activity in RNA to direct protein synthesis (Fig. 2); ▲, messenger activity in RNA to direct f-met-tRNA binding (Fig. 3).

Figure 4 summarises the above results obtained in the presence of rifampicin. After the addition of rifampicin at 10 min of infection, when there was extensive synthesis of phage RNA, there was a slight increase of functional activity due to the residual synthesis of RNA expected from the mode of action of rifampicin. Thereafter, a rapid decay of functional activity in directing total protein synthesis and also initiation factor-dependent f-met-tRNA binding took place. Both decay curves are almost parallel and give an estimate of the functional half-life of T7 early mRNA as about 6.5 min at 30° C. On the other hand, the acid-insoluble and hybridisable amounts of the RNA remained stable.

A loss of structural integrity in T7 early mRNA was found by electrophoresis of the RNA in polyacrylamide gel containing agarose. This method gave a much better resolution for the T7 early mRNA species⁴ than the procedure previously used. RNA extracted from ultraviolet-irradiated D10 F⁻ cells infected with T7 *am193* was labelled with ³H-uridine. Portions of these RNA samples were used for hybridisation experiments (Fig. 1b). Electrophoretic patterns in Figs 5 and 6 show that T7 early mRNAs were partially degraded as time progressed in the absence (Fig. 5) and presence (Fig. 6) of rifampicin. It seems that more smaller size RNAs were produced, at the expense of full size RNAs. Since total radioactivity in the RNA samples taken at different times in the presence of rifampicin was almost the same (see Fig. 1a and b) and relatively large RNAs remained after a long time, it is unlikely that an extensive nucleolytic degradation had taken place. Figure 5 and 6 show that the largest species of T7 early mRNA, gene 1 messenger, over-

lapping with the marker 23S rRNA (see ref. 4) at 10 min (Figs 5a and 6a) shifts to a slightly smaller size as time proceeds (Figs 5 and 6-d).

Our results show that structural integrity and functional activity of T7 early mRNA are closely related. Loss of structural integrity, not detected by measuring chemical amount or hybridisability, plays an important role in messenger function. This warns that hybridisable radioactivity in mRNA is not necessarily a measure of functional activity of the mRNA. Our results also explain why only late proteins are produced in the presence of chemically stable early mRNA in late T7 infection.

Whereas in *E. coli*, mRNAs appear to have identical or similar decay half-lives in functional activity and chemical amount (see ref. 5), a similar difference between hybridisability and functional stability in mRNA was found in single-stranded DNA phage S13 (ref. 6). Messenger RNAs of other phages, ΦX174 (ref. 7) and M13 (ref. 8), were shown to have relatively long half-lives in terms of hybridisability. It would be interesting to know whether these phage mRNAs show a difference between functional and chemical stability.

At the moment we have no direct information as to why T7 early mRNA is chemically stable. Since host mRNA (at least tryptophan mRNA) decays normally in T7-infected cells², T7-directed modification of general mRNA degradation system of the host is unlikely. It is conceivable that T7 mRNA may have structural features, such as extensive base-pairings

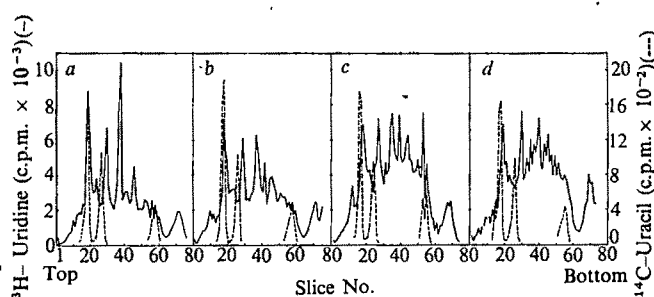


FIG. 5 Polyacrylamide-agarose gel electrophoretic pattern of T7 *am193* mRNA. The ³H-labelled RNA samples, used in the hybridisation experiment (Fig. 1b), were analysed by gel electrophoresis. RNA samples (about 1.7×10^5 c.p.m. per sample) were mixed with ¹⁴C-labelled total *E. coli* RNA and subjected to electrophoresis in 2.5% acrylamide-0.5% agarose gel for 1.5 hours at 5 mA per gel^{4,13}. Gels were frozen and sliced into 1 mm pieces. Radioactivity of ³H in each slice was counted⁴. The direction of electrophoresis was from left to right. a, T7 *am193* RNA from ultraviolet-irradiated cells 10 min after infection; b, 15 min; c, 20 min; d, 25 min; —, ³H-labelled T7 *am193* RNA; ---, ¹⁴C-labelled marker *E. coli* RNA, 23S, 16S, and 4S, from left to right.

or special base sequences, such that host mRNA degradation enzyme(s) fail to attack the phage mRNA. An endonucleolytic enzyme, identical or similar to RNase III⁹, is required to process a large single piece of RNA synthesised *in vitro* by *E. coli* RNA polymerase on T7 DNA template. Dunn and Studier¹⁰ showed that this large size RNA, a transcript of entire T7 early genes, can be cleaved to yield several pieces of smaller size RNAs corresponding to the size of *in vivo* T7 early mRNAs. Since RNase III is a double stranded RNA-specific endonuclease, this finding indicates some degree of base pairing in T7 early mRNA.

A preliminary experiment using wild-type T7 phage, instead of T7 *am193*, indicated that the functional activity of both early and late mRNA decays rapidly. Recently, Hagen and Young¹¹ reported that T7 lysozyme mRNA (transcript of one of the large genes) is unstable in directing *in vitro* synthesis of lysozyme.

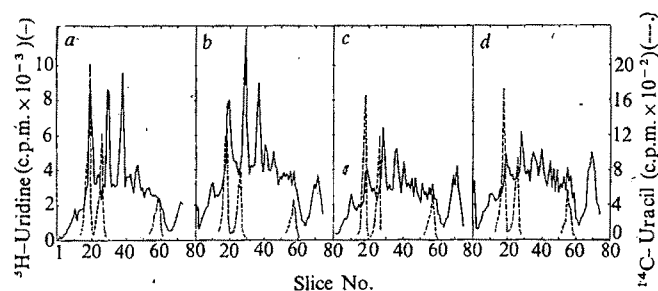


FIG. 6 Polyacrylamide-agarose gel electrophoretic pattern of T7 *am193* mRNA after rifampicin treatment. RNA samples were as used in the hybridisation experiment shown in Fig. 1b. Electrophoresis was carried out as described in the legend for Fig. 5. a, T7 *am193* RNA from ultraviolet-irradiated cells 12 min after infection (rifampicin was added at 10 min); b, 15 min; c, 20 min; d, 25 min; —, ^3H -labelled T7 *am193* RNA; ---, ^{14}C -labelled marker *E. coli* 23S, 16S, and 4S, from left to right.

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Hydrophobic bonding and accessible surface area in proteins

THE hydrophobic bond is the term used by Kauzmann¹ to describe the gain in free energy on the transfer of non-polar residues from an aqueous environment to the interior of proteins. This has been accepted as one of the major forces involved in the folding of proteins. The exact origin of the energy of the hydrophobic bond is controversial², but empirical values have been derived for 10 protein residue side chains by Nozaki and Tanford³ who measured the solubility of amino acids in the organic solvents ethanol and dioxane.

For an atomic group in a protein to interact with the solvent it must be able to form Van der Waals contracts with water molecules. The concept of accessible surface area

has been introduced by Lee and Richards⁴ to describe quantitatively the relationship of proteins to solvent. The accessible surface area of an atomic group is defined as the 'area on the surface of a sphere radius R , on each point of which the centre of a solvent molecule can be placed in contact with the atom without penetrating any other atoms of the molecule'. R is the sum of the Van der Waals radii of the atom and the solvent molecule.

In Fig. 1, the hydrophobicity of each of the 10 protein residue side chains is plotted against its accessible surface area. To derive the hydrophobicity of a side chain Nozaki and Tanford subtracted the value for glycine from that of the amino acid. I calculated the accessible surface areas of protein residue, X , from the accessibilities given by Lee and Richards⁴ for X in tripeptide Ala- X -Ala, where the main chain is in an extended β conformation and the side torsion angles antiplanar. The values for the side chains in Fig. 1 are the accessible surface area of the residue minus the accessible surface area of glycyl (82 Å²).

From Fig. 1 we see that for the non-polar side chains Ala, Val, Leu and Phe there is a linear relationship between hydrophobicity and accessible surface area; the correlation coefficient is 0.998 and the slope equivalent to 22 calorie Å⁻². For the side chains with hydroxyl groups Ser, Thr and Tyr there is a similar linear relationship with a correlation coefficient of 0.998 and a slope equivalent to 26 calorie Å⁻². For residues with similar accessible surface areas those with a polar group are about 1 kilocalorie less hydrophobic than those with only non-polar groups. In aqueous solution the OH, NH, N and S groups will form hydrogen bonds with water. But on transfer to ethanol or dioxane these solute-solvent hydrogen bonds are significantly weakened⁵ and there is therefore a loss of free energy (1-2 kilocalorie per polar group is a reasonable estimate). In proteins this loss does not occur: ~92% of polar groups buried in the interior of proteins form hydrogen bonds. Thus the hydrophobicity of polar side chains will be greater than the values in Fig. 1. A 'correction' of 1-2 kilocalorie per polar group makes the polar residues as hydrophobic as non-polar residues. (The histidine side chain has two polar groups.) This implies that, if hydrogen-bonded, oxygen and nitrogen atoms are hydrophobic; they could, for example, be favourably accommodated in the interior of membranes. This result is related to the finding of Lee and Richards⁴ that when lysozyme, RNase S and myoglobin go from an extended chain to the folded conformation, the reduction in accessible surface area is almost the same for polar and non-polar atoms. Methionine is a little less, and tryptophan a little more, hydrophobic than this argument and the linear relationship illustrated in Fig. 1, would suggest.

In conclusion, we can say that as a good approximation the hydrophobicity of residues in proteins is 24 calorie per Å² of accessible surface area. This value is applicable to protein folding, subunit contacts and the interaction of small molecules with enzymes and immunoglobins. For example the formation of the $\alpha_1\beta_1$ contact in haemoglobin reduces in each monomer the surface area accessible to water by ~900 Å² (my unpublished results). This implies that the hydrophobic contribution to the free energy of dimer formation is 43 kilocalorie. The formation of the contacts that cause the reduction in accessible surface area when proteins fold or aggregate involves large conformational entropy losses^{6,7} and appreciable steric strain (Szabo and Karplus, personal communication) which substantially reduces the figure for the free energy.

The value given here for the hydrophobicity of protein surfaces confirms Kauzmann's hypotheses¹ that the entropic term that stabilises protein aggregation and the binding of small molecules is mainly a result of hydrophobic bonding, and suggests that only relatively small contact areas are necessary for stability. In haemoglobin ~12% of the ac-

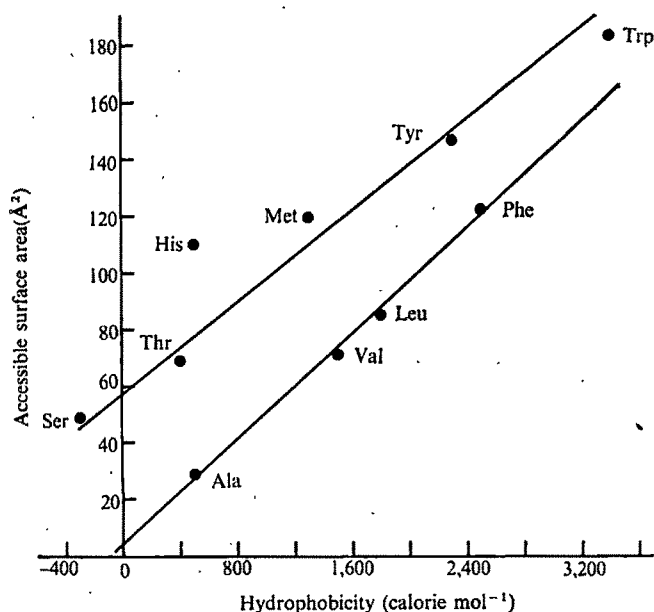


Fig. 1 Accessible surface areas of residue side chains (see text) plotted against hydrophobicity (free energy change for the transfer from 100% organic solvent to water³).

cessible surface area of each monomer is covered by the $\alpha_1\beta_1$ contact and $\sim 6\%$ by the $\alpha_1\beta_2$ contact.

In a discussion of the general theory of hydrophobic bonding Hermann⁸ deduced and found a linear relationship between the logarithm of the solubility of hydrocarbons and the surface area of the cavity they form in water. This latter term is equivalent to the accessible surface area of the hydrocarbons.

I thank my colleagues for critical discussion.

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Insulin/proinsulin, a new crystalline complex

SINCE the first crystallisation of proinsulin in this laboratory¹, we have tried to grow crystals appropriate for detailed X-ray crystal structure analysis. None of the proinsulin crystal forms prepared proved ideal², and we attempted to cocrystallise insulin and proinsulin. Such crystals, if both components were in ordered array, could provide a basis for the determination of the structure of proinsulin by the use of rigid-body vector search methods^{3,4} using the known stereochemistry of insulin⁵.

Proinsulin is found in crystalline native insulin preparations⁶. It is common to find proteins from inhomogeneous fractions incorporated as impurities into crystals of the

dominant component⁷. Very similar protein molecules, for example, β_1 and β_2 lactoglobulin⁸ often form mixed crystals. These are defect solids, with all crystallographically equivalent positions occupied, but by molecules of different kinds. Steiner⁹ has reported the preparation of what he seems to claim are mixed crystals of insulin and proinsulin of this type. Mixed crystals are not cocrystals if, by this we imply structures with each separate protein ordered by the full symmetry of the space group nor, because of disorder, are they useful for X-ray crystal structure analysis.

We report here the isolation and preliminary X-ray study of a 1:1 crystalline complex of insulin and proinsulin which seems to be a true cocrystalline form.

Cocrystallisation studies were made with both beef and pork hormone/prohormone pairs using the molar ratio 1:1 exclusively. Heterogeneous pairs were not studied. Separate insulin and proinsulin solutions of initial molarities 1.4 mM were prepared by dissolving each protein in 50 μ l of a solution containing 0.05 M sodium citrate, 30 mg ml⁻¹ sodium chloride, and, where used, 0.836 mg ml⁻¹ of zinc chloride and 3 mg ml⁻¹ of *m*-cresol. The final pH was adjusted to 7.25 by addition of 0.5 N HCl. Both proteins crystallise at this pH in the presence of *m*-cresol and zinc; insulin as diamond-shaped plates, proinsulin as tetragonal prisms with pyramidal termination. Hormone and pro-hormone solutions were warmed to 50° C with stirring, then mixed and kept at 50° C for a few minutes. Three vials of bovine material containing cresol and zinc were prepared. All gave a precipitate on cooling to room temperature. Protein-free crystallisation medium was added (with rewarming) to provide a series with final volumes of 130, 150 and 180 μ l respectively. All vials were then kept at room temperature. Small, well-formed crystals began to appear after 2–4 d. The vials were then stored at 2° C. The crystals continued to grow for 1–2 weeks.

All preparations gave crystals. Only tiny, unidentified crystals, 50–60 μ m long grew in the absence of *m*-cresol and/or zinc. The crystals in both pork and beef insulin—

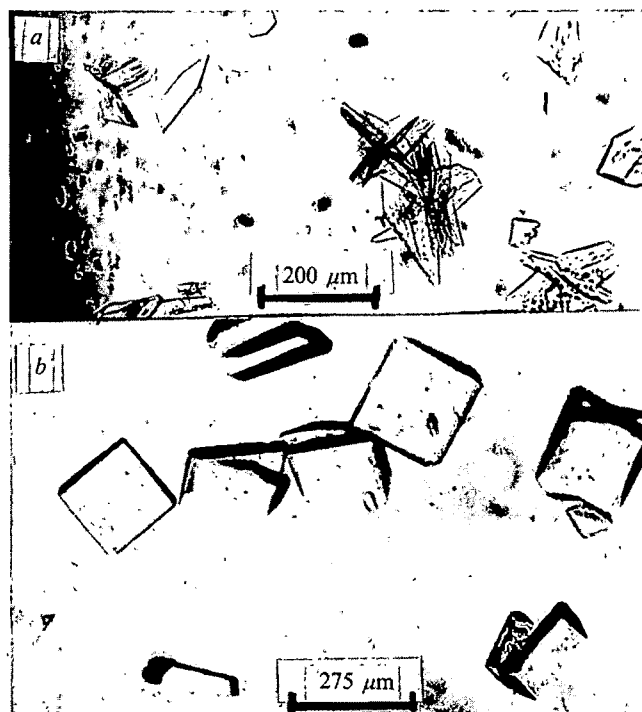


Fig. 1 a, 1:1 crystalline complex of porcine insulin/proinsulin; b, 1:1 crystalline complex of bovine insulin/proinsulin.



Fig. 2 Precession photograph of a bovine insulin/proinsulin complex, 72 h exposure ($\mu = 2.5^\circ$).

proinsulin preparations are clear, colourless plates or wedges which extinguish sharply along the face diagonals under polarised light. The porcine crystals (Fig. 1a) were very thin, and less well formed than the bovine crystals which measured on average $190 \mu\text{m} \times 190 \mu\text{m} \times 30 \mu\text{m}$ (Fig. 1b).

Bovine insulin/proinsulin crystals were mounted in sealed thin-walled quartz capillaries and X-ray precession photographs taken using unfiltered $\text{CuK}\alpha$ ($\lambda = 1.5418 \text{ \AA}$) radiation from a Jarrel-Ash microfocus tube with a pinhole collimator. The crystals turned opaque after less than 30 h exposure to X rays at 20°C (crystals dissolve in their mother liquor at 21°C). Cooling to 15°C retards but does not halt radiation damage.

Useful diffraction patterns were obtained only with the X-ray beam approximately normal to the main face. From still photographs and two small angle ($\mu = 2.5^\circ$) precession films (Fig. 2) the unit cell dimensions were determined to be $257 \text{ \AA} \times 125 \text{ \AA} \times 83 \text{ \AA}$. It was not possible to determine the space group. The two longest dimensions lie along the face diagonals of the crystal.

The unit cell volume is $2.67 \times 10^6 \text{ \AA}^3$. The calculated weight fraction of protein based on a partial specific volume of 0.72 (ref. 1) for both insulin and proinsulin, and a crystal density of 1.20 g cm^{-3} (ref. 1) is 0.52. This is 10^6 daltons, equivalent to twelve hexamers each of insulin and proinsulin per unit cell.

The crystals were analysed by polyacrylamide disc gel electrophoresis, a procedure valid only if the crystals used are identical with the crystals studied by X-ray diffraction. After extensive microscopic survey the larger crystals were removed from those two vials used for X-ray study. These crystals (approximately 100) weighed about $10 \mu\text{g}$. They were washed twice with separate 1 ml portions of protein-free buffer (that is, about 2.4×10^6 times their volume) to remove all non-crystalline protein on their surfaces.

The relative amounts of insulin and proinsulin in the crystals were then determined: The crystals were dissolved in Davis standard buffer (pH 6.7) and applied to gels prepared according to Davis' conditions¹⁰. Similar amounts of insulin and proinsulin were each applied to separate gels as positional standards. Gels were run for 50 min at 8 mA per gel, then stained with Buffalo Black NBR and scanned with a Canaco model E microdensitometer. The gel containing the dissolved crystals showed two bands at the positions of the insulin and proinsulin standards. The relative darkening of the two bands was identical, within 3%, to that on a standard gel run with an equimolar solution of insulin and proinsulin of approximately the same protein concentration.

To verify these findings we wished to use other methods of analysis. Amino acid analysis is insensitive and inappro-

priate because of the close similarity in amino acid composition between the two proteins. An alternative analytical procedure involving separation on a column and subsequent determination of insulin and proinsulin from measurement of absorbance was suggested to us by Steiner (personal communication, 1972) as more reliable than disc gel electrophoresis. It would unfortunately have required the preparation of prohibitively large amounts ($\sim 1 \text{ mg}$) of insulin/proinsulin crystalline material.

We finally chose to calibrate the protein-dye interaction using eleven solutions of insulin/proinsulin with molar ratios varying from 0.5 to 2.0 ($1 \mu\text{g} \mu\text{l}^{-1}$ of protein). The plot of molar ratios of samples against the relative insulin/proinsulin band darkening effects is linear within the range studied and confirms our earlier finding. The insulin/proinsulin ratio is 1:1 within less than 5% maximum error.

We have prepared and characterised a 1:1 crystal complex with approximately twelve hexamers each of insulin and proinsulin per unit cell. As both proteins crystallise separately under the conditions used, there must be strong interactions leading to the new complex between insulin and proinsulin.

The crystals described here differ from any prepared by Steiner. The initial conditions for crystallisation are different (proinsulin has not been crystallised under the conditions used by Steiner). Steiner seems to imply that his crystals are mixed crystals (conforming, at least at low proinsulin ratios, to the dominant rhombohedral insulin form) containing one proinsulin molecule per eleven insulin molecules (8%) that is, on the average one proinsulin per two hexamers, or respectively one, two or three proinsulin molecules per hexamer.

We know of no studies which suggest that insulin and proinsulin form mixed dimers and hexamers in solution. It is difficult to accept the kinetics and equilibria required by the Steiner model—exchanges between homogeneous dimers and hexamers of insulin and proinsulin before crystallisation followed by prompt disassociation and complete reassociation into wholly insulin and proinsulin aggregates in the gel. The precise identity of the Steiner crystals must await adequate characterisation.

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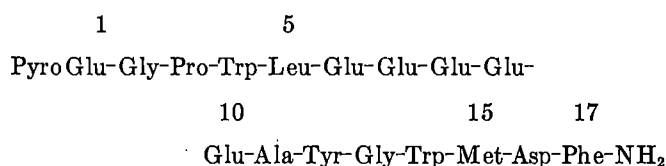
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pH-dependent conformational change of gastrin

GASTRIN is a polypeptide hormone with a broad spectrum of biological activities, most notably the stimulation of secretion of gastric acid^{1,2}. It is produced primarily in the pyloric gland area of the lower segment of the stomach, the antrum, which does not secrete acid^{1,2}. When carried by the blood to the upper segment of the stomach, the fundus, gastrin stimulates secretion of acid by the oxyntic glands.¹ Although release of gastrin into the blood stream is mediated by stimuli from the nervous system, its release is strongly inhibited when the antral mucosa is bathed in acid². Therefore, the secretion of the hormone is under negative feedback regulation, with resultant control of stomach acidity.

Human gastrin is a polypeptide composed of seventeen amino acid residues³:



It is found in two forms: without (gastrin I) and with (gastrin II) a sulphate esterified to the hydroxyl group of tyrosine-12. The amino terminus is blocked by the formation of a pyroglutamyl group, and the carboxyl-terminus is blocked as an amide. The carboxyl-terminal tetrapeptide amide fragment (residues 14 to 17) has the full range of physiological activities of the intact hormone⁴. No function for the amino-terminal tridecapeptide (residues 1 to 13) has been demonstrated. A striking structural feature of this portion of the hormone is the sequence of five glutamic acid residues (residues 6 to 10). Synthetic polymers of *L*-glutamic acid have been shown to undergo a transition from random coil to the α -helical conformation as the pH is lowered^{5,6}.

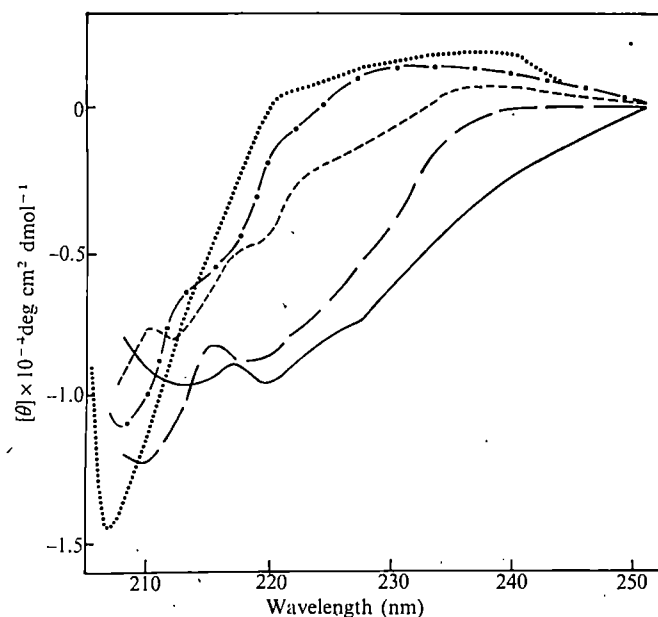


Fig. 1 Circular dichroic spectra of gastrin I at varying pH values. pH: ····, 8.10; —○—, 6.68; ----, 4.85; —, 3.91; —×—, 2.81. Aliquots of a stock solution (1.0 mg ml⁻¹ in 0.05 M NH₄HCO₃) were added to very dilute HCl solutions to give a gastrin concentration of 0.02 mg ml⁻¹ at the indicated pH values. Circular dichroic spectra were measured with a Beckman CD spectrophotometer equipped with a Hewlett-Packard 5480A signal analyser. Measurements of samples in a 1.0 cm path length cell were made at 22°C. All spectra are averages of a minimum of 150 scans. Ellipticities ([θ]) have been expressed in units of degree cm² per decimol of amino acid.

Since the antrum of the stomach is normally bathed in solutions with pH values in this same acidic region, it seemed possible that a similar pH-dependent conformational change of gastrin was involved in the inhibition of its release by acid. As a first step in testing this hypothesis, the circular dichroic spectra of gastrin as a function of pH were studied as probes of its conformations.

The circular dichroic spectra of synthetic human gastrin I (referred to here as gastrin, obtained from ICI, Cheshire,

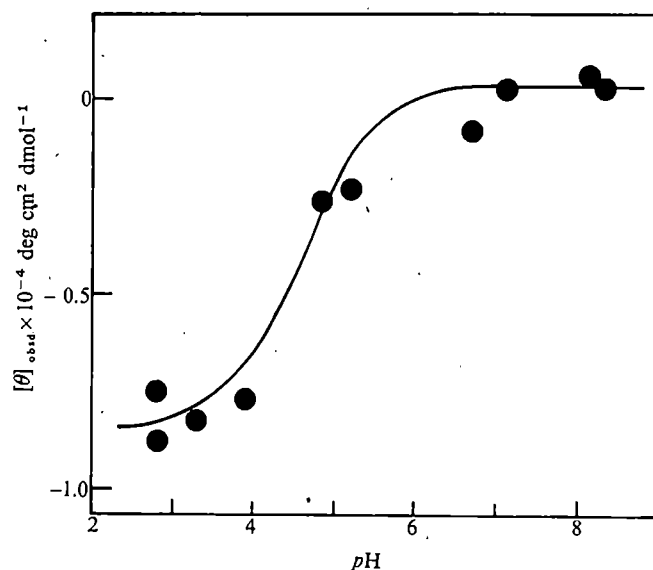


Fig. 2 Variation of the observed ellipticity of gastrin I at 222 nm with pH. Points were obtained experimentally. The curve is theoretical, and was calculated from equation (1).

England) at varying pH values is presented in Fig. 1. The spectrum measured near neutrality (for example at pH 8.10) has a small positive ellipticity in the 220 to 240 nm range and large negative ellipticity at shorter wavelengths. Qualitatively it is very similar to the spectrum of the random coil forms of poly-*L*-glutamic acid⁷ and poly-*L*-lysine^{7,8}. The circular dichroic spectrum of gastrin in acidic solution (for example at pH 2.81) has two peaks of negative ellipticity at 212 and 220 nm. These negative peaks are in approximately the same positions as the two peaks of negative ellipticity which characterise the α -helical conformations of poly-*L*-glutamic acid⁷ and poly-*L*-lysine^{7,8}. It is interesting that a maximum in negative ellipticity ([θ]) centred near 217 nm which is characteristic of β -structure is absent from all spectra (Fig. 2), indicating the absence of substantial amounts of β -structure. Unfortunately, spectra recorded below 210 nm are not sufficiently free of noise to allow detection of an isosbestic point which would indicate the absence of a conformational intermediate between the random coil and α -helical forms. This low signal-to-noise ratio was a result of the low concentration of gastrin used, which was dictated by its low solubility in acidic solution.

The random coil and β -structure have the same ellipticity at 222 nm⁸. Therefore, the magnitude of observed ellipticity [θ]_{obs} at this wave length (all measurements and values of [θ] used below are at 222 nm) can be used to approximate the helical content of a protein or polypeptide at any given pH. The variation of [θ]_{obs} with pH can be used as a measure of formation and breakdown of α -helical structures with change of pH. A plot of the observed ellipticity at 222 nm as a function of pH is presented in Fig. 2.

As a first approximation in attempting to derive a theoretical treatment to fit the dependence of [θ]_{obs} on pH, two simplifying assumptions have been made. First, the conformational change and resultant change of [θ]_{obs} depends on a single ionisation. Second, only the complete random coil, and the partial α -helical conformations may exist. The points in

Fig. 2 can be fitted to a theoretical curve described by equation (1), where $[\theta]_{\text{obsd}}$ is the

$$[\theta]_{\text{obsd}} = [\theta]_{\alpha \text{ obsd}} \left[\frac{[H^+]}{K_{\text{app}} + [H^+]} \right] + [\theta]_{R+\beta \text{ obsd}} \left[\frac{K_{\text{app}}}{K_{\text{app}} + [H^+]} \right] \quad (1)$$

observed ellipticity; $[\theta]_{\alpha \text{ obsd}}$ is that fraction of the observed ellipticity attributable to α -helical structures, and $[\theta]_{R+\beta \text{ obsd}}$ is that fraction attributable to random coil and β -structures. The terms $[H^+]/(K_{\text{app}} + [H^+])$ and $K_{\text{app}}/(K_{\text{app}} + [H^+])$ define the mol fraction of gastrin in the conformation which predominates under acidic or neutral conditions respectively. The curve shown in Fig. 3 has been calculated to give the best fit to the experimental points with the following values: $K_{\text{app}} = 2.51 \times 10^{-5}$ ($pK_{\text{app}} = 4.6$), $[\theta]_{R+\beta \text{ obsd}} = +300$, and $[\theta]_{\alpha \text{ obsd}} = -8,300$. The apparent pK_a is similar to that for the ionisation of a γ -carboxyl of a glutamic acid residue ($pK_a = 4.4$)⁹. The value of $[\theta]_{R+\beta \text{ obsd}}$ is slightly more positive than $[\theta]_R = -2,780$ and $[\theta]_{\beta} = -2,670$ which have been derived from studies of five globular proteins¹⁰, and within the limits of $[\theta]_R = +3,900$ and $[\theta]_{\beta} = -13,800$ found for poly-L-lysine⁸. Thus, the value of $[\theta]_{R+\beta \text{ obsd}}$ is compatible with gastrin being a random coil in neutral solution. While the theoretical treatment of the pH-dependence of $[\theta]_{\text{obsd}}$ defined by equation (1) gives a good fit to the experimental data, it is probably an oversimplification. Additional factors may be considered which, if included, might give a more precise description of this helix to random coil transition. Their involvement is not obvious in the experimental data, however (Fig. 2).

As a first approximation the fraction of the gastrin molecule which assumes the α -helical conformation in acidic solution can be calculated from equation (2)⁸,

$$\text{fraction } \alpha\text{-helix} = \frac{[\theta]_{\alpha \text{ obsd}} - [\theta]_{R+\beta \text{ obsd}}}{[\theta]_{\alpha} - [\theta]_{R+\beta \text{ obsd}}} \quad (2)$$

where $[\theta]_{\alpha}$ is the ellipticity of a completely helical structure. If a value of $[\theta]_{\alpha} = -35,000$ ⁸ is used, 0.24 of the gastrin molecule, or 4.1 amino acids, assume an α -helical conformation in acidic solution. If the value of $[\theta]_{\alpha} = -31,500$ ¹⁰ is used, 0.27 of the gastrin molecule, or 4.6 residues, participate in the α -helix.

Equation (2) is based on the assumption that ellipticity ($[\theta]_{\alpha}$) is independent of the length of the helix. In fact,

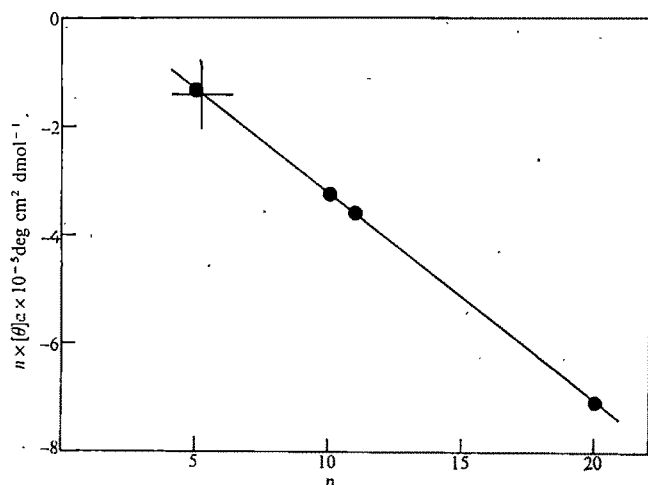


Fig. 3 Ellipticity per decimol of helical segment (that is, $[\theta]_{\alpha} \times$ the number of amino acids per helical segment) plotted as a function of the number of amino acids in the helix. Values of $n \times [\theta]_{\alpha}$ for 5, 10, and 20 residues were calculated from the ellipticities of polymers of alanine^{10,11}; $n \times [\theta]_{\alpha}$ for 11 residues is an average value, calculated from the observed ellipticities of several globular proteins¹¹.

calculated circular dichroism spectra of polymers of alanine¹¹ indicated that $[\theta]_{\alpha}$ decreases with polypeptide chain length, most noticeably for short helical segments^{10,11}. The simplest way to correct for this deviation is by a graphical method. If the ellipticity per decimol of helical segment (that is, $[\theta]_{\alpha} \times n$, where n is the number of amino acids per helical segment) is plotted as a function of the number of amino acids in the helix (n) a linear relationship is obtained (Fig. 3). If only one segment of helix is present in gastrin at low pH, then the ellipticity per decimol of gastrin (that is, $[\theta]_{\alpha \text{ obsd}} \times 17 = 1.41 \times 10^4$) when included in this plot corresponds to $n = 5.2$ residues in an α -helix.

In view of the uncertainty involved in calculating by the above methods the number of residues which assume the helical conformation and the uncertainty inherent in the experimental data, it is reasonable to conclude that the α -helical structure of gastrin contains 5 ± 1 amino acid residues. Since polymers of L-glutamic acid are known to undergo a similar transition from random coil to α -helix, the amino acid residues of gastrin most likely to be involved in α -helix formation are the five sequential glutamic acids (6 to 10).

Gastrins from different species do not always contain the five consecutive glutamate residues. In gastrin from cat, cow and sheep, glutamate 10 is replaced by alanine², and in dog gastrin glutamate 8 is replaced by alanine². Polymers of L-alanine have been studied extensively by X-ray diffraction and found to be in the α -helical form¹². Also, on the basis of energy computations, alanine has been recognised as an amino acid which promotes α -helix formation¹³. Furthermore, in the tertiary structures of seven globular proteins, alanine has been found to have a greater than random probability of participating in an α -helix¹⁴. Thus, the substitution of alanine for glutamic acid in some species is not likely to prevent helix formation, although this has yet to be demonstrated.

Release of gastrin from the pyloric gland area of gastric mucosa of the stomach is inhibited when this tissue is bathed in acid². If gastric acid could interact with gastrin within this tissue, the partial α -helical conformation would be induced. For any of a variety of possible reasons, the conversion of gastrin to the α -helical conformation might prevent its secretion. Thus, the pH-dependent conformational change of gastrin may play a physiological role in the mechanism of acid inhibition of its release.

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Stimulation of calcitonin synthesis and release *in vitro* by calcium and dibutyryl cyclic AMP

It has been proposed that the physiological action of calcitonin is to prevent or diminish postprandial hypercalcaemia¹. This is accomplished by inhibition of bone resorption², of the tubular reabsorption of calcium³, and of the intestinal absorption of calcium⁴. Secretion or release of the hormone by parafollicular cells of the thyroid gland is mediated by calcium⁵⁻⁷ and by gastrointestinal hormones including gastrin and pancreozymin^{8,9}. Secretion is apparently influenced by the adenylate cyclase system^{7,10} and hormonal regulation presumably is mediated by cyclic adenosine 3', 5'-monophosphate (cyclic AMP).

We have carried out studies to determine whether calcium and dibutyryl cyclic AMP influence synthesis as well as release of calcitonin. For this purpose slices of porcine thyroid tissue were incubated for 3 h in Krebs-Ringer phosphate buffer. After incubation calcitonin in the incubation medium and in thyroid tissue extracts was determined by radioimmunoassay^{7,11}. As compared with unincubated tissue, there was a three-fold and highly significant mean increase in total calcitonin when tissues were incubated in the presence of a high concentration of calcium, 5.0 meq l⁻¹ (Table 1). Calcium, 7.5 meq l⁻¹, produced mean increases of 75% in the quantity of hormone released into the medium and of 58% in the total amount of hormone as compared to control samples incubated with no calcium in the medium (Table 2). Dibutyryl cyclic AMP, 10 mM, produced mean increases of 28% in the amount of calcitonin released into the medium and of 20% in the total quantity of hormone as compared with control samples incubated with no cyclic nucleotide in the medium (Table 3). These results, which are representative, show that thyroid slices readily synthesise calcitonin during a 3 h period of incubation and that calcium and dibutyryl cyclic AMP produce modest but significant mean increases in calcitonin synthesis as well as secretion.

TABLE 1 Effects of time and calcium* on synthesis and release of calcitonin *in vitro*.

Incubation (h)	Tissue	Calcitonin Medium (ng)	Total	No.
0	301 ± 46	—	301 ± 46	9
3	461 ± 80	752 ± 66	1213 ± 98	9
	NS		<0.001	

Slices of porcine thyroid glands weighing 100 mg (±5%) were used. Half of them were incubated in 4 ml Krebs-Ringer phosphate buffer, pH 7.4, for 3 h at 37°C under 100% O₂. Slices from a single gland were used in each experiment. Magnesium concentration was 3.1 meq l⁻¹ and glucose concentration was 1 mg ml⁻¹. After incubation, the slices were freeze dried and extracted three times with butanol-acetic acid and water (75:7.5:21)¹². The extracts were combined, freeze dried and taken up in the buffer used for the immunoassay⁷. The unincubated slices were similarly treated. Calcitonin in extracts and medium was estimated by radioimmunoassay in quadruplicate at two different dilutions^{7,11}.

Results are given as ng and are expressed as mean ± one s.e. Student's *t* test was used to determine statistical significance of differences.

* The incubation medium contained 5 meq l⁻¹ of calcium.

Very little is known about the effects of long term stimulation by calcium on either the synthesis or secretion of calcitonin. Gittes *et al.*¹³ showed that calcitonin content in thyroid glands of rats could be reduced by 65% with hypercalcaemia produced by the administration of calcium. Conversely, they demonstrated progressive accumulation of calcitonin content of the thyroid glands with hypocalcaemia produced by parathyroidectomy. Indeed, in the parathyroidectomised animals values of calcitonin content as high as

TABLE 2 Effects of calcium on the synthesis and release of calcitonin *in vitro*.

[Ca ²⁺] (meq l ⁻¹)	Tissue	Calcitonin Medium (ng)	Total	No.
None	525 ± 79	724 ± 59	1249 ± 48	10
7.5	717 ± 70	1262 ± 178	1979 ± 168	10
	NS	< 0.01	< 0.001	

twelve-fold that of values in control animals were found after 14 weeks. It was concluded that the thyroid gland is much better adapted to secrete calcitonin in response to acute hypercalcaemia than in response to chronic hypercalcaemia.

There is little information currently available concerning the effects and mechanism of action of cyclic AMP to stimulate the synthesis of protein hormones. In pulse-chase experiments, Tanese *et al.*¹⁴ showed that the incorporation of ³H-leucine into insulin and proinsulin and subsequent release

TABLE 3 Effects of dibutyryl cAMP on synthesis and release of calcitonin *in vitro*.

Dibutyryl cAMP (mM)	Tissue	Calcitonin Medium (ng)	Total	No.
None	531 ± 78	1255 ± 85	1786 ± 134	10
10	577 ± 84	1580 ± 81	2156 ± 108	10
	NS	< 0.02	< 0.05	

The concentration of calcium in the incubation medium was 2.5 meq l⁻¹.

of the hormone and prohormone by isolated islet cells of rats *in vitro* are increased by dibutyryl cyclic AMP. Similarly, Abe and Sherwood¹⁵ demonstrated that incorporation of ¹⁴C-leucine into parathyroid hormone and release by bovine parathyroid tissue *in vitro* is also augmented by dibutyryl cyclic AMP. These studies together with our own suggest that cyclic AMP may modify synthesis as well as secretion of protein hormones.

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Relationship between measles and canine distemper viruses determined by delayed type hypersensitivity reactions in dogs

MEASLES virus, canine distemper virus and rinderpest virus are classified as members of a subgroup of paramyxoviruses with various interrelationships (reviewed in ref. 1). In some species and in some combinations the viruses may provide a measure of protection against virulent heterologous challenge. This has been the premise for using measles virus vaccine to protect pups against canine distemper at an age when passive maternal immunity often interferes with canine distemper vaccination²⁻⁵.

The principal protection against natural primary measles infection is considered to be cell-mediated immunity^{6,7}, and so it seemed likely that this mechanism also protects against canine distemper. Because of the relationship between the two viruses, we reasoned that measles virus should stimulate the response against distemper. We have tested this hypothesis with dogs to determine whether delayed hypersensitivity reactions could be elicited with both homologous and heterologous antigens.

We used the Edmonston strain of measles virus, which had been through twenty-four passages in human kidney

TABLE 1 Delayed hypersensitivity reaction in dogs sensitised with measles virus after challenge with canine distemper virus

Sensitising antigen	Challenge antigen	
	Canine distemper	NL-DK-1
Measles virus	3/4*	0/4
Canine distemper	2/2	0/2
NL-DK-1 (control)	0/2	0/2

* Numerator indicates number of positive reaction sites, denominator indicates number of dogs tested.

culture, twenty-eight in human amnion culture, six in primary dog kidney culture and two in an established dog kidney cell line (NL-DK-1). The canine distemper virus used was the Snyder Hill strain which had been modified by twenty-eight passages in the NL-DK-1 cell line. The history and tissue culture procedures used for this cell line⁸ and for the viruses⁵ have been described previously. To prepare the sensitising antigens, a large sample of cells growing in Roux bottles was divided into three portions. One portion was infected with measles virus, another with canine distemper virus and the remainder was left uninfected. At the appropriate time, antigens were collected and frozen at -50°C . The live measles viral antigen had a virus titre of $10^{6.26}$ TCID₅₀ ml⁻¹, while that of the live canine distemper viral antigen was $10^{4.63}$ TCID₅₀ ml⁻¹.

Cells used to prepare the skin test reagents were handled similarly except that after collection the virus was concentrated by differential centrifugation. Fluids were centrifuged at 2,000 r.p.m. in a 267A head in a model BE International centrifuge for 15 min followed by centrifugation at 55,000 r.p.m. in a SW 27 head on a model L3 Beckman centrifuge at 5°C for 120 min. After resuspension of the pellet in Hanks balanced salt solution, virus had been concentrated about forty-fold. The cell control antigen was handled in an identical manner. The resuspended concentrates were then treated with Tween-80 and ether as described by Norrby⁹ which served to inactivate the viral antigens.

Our test procedure determined by preliminary experiments consisted of a single inoculation with a sensitising antigen (live virus) followed 10-30 d later by test inoculations with skin test reagents. The sensitising inoculations of 5.0 ml were given intramuscularly in the hind legs. Test inoculations of 0.2 ml were given subcutaneously in the inside surface of the ears. Up to three different antigens were inoculated at separate sites in each ear with inoculations being repeated bilaterally. Dogs had to be kept at a minimum temperature of 20°C , as at a lower temperature cold ears interfered with the test and gave false negative reactions. (This is important if experiments are conducted with animals maintained in the open.) Immediately before each inoculation, blood samples were tested for neutralising antibodies against both canine distemper and measles viruses⁵. Samples collected at the first inoculation were tested to determine that dogs had not been exposed previously. Samples collected at the second inoculation were tested to ensure that each dog had shown an appropriate response to the sensitising antigen alone. Dogs were examined 4, 24, 48, 72 and 96 h after subcutaneous challenge. No reactions indicative of an immediate hypersensitivity response were observed at 4 h. The first positive reactions, palpable at 24 h, consisted of a small region characterised by oedema and induration which had generally increased in size at 48 h. Lesions were not characterised by erythema. At 72 and 96 h the lesions were smaller but still palpable and gradually disappeared in about a week. In some experiments, dogs were rechallenged and the lesions tended to be larger and occasionally exhibited central necrosis.

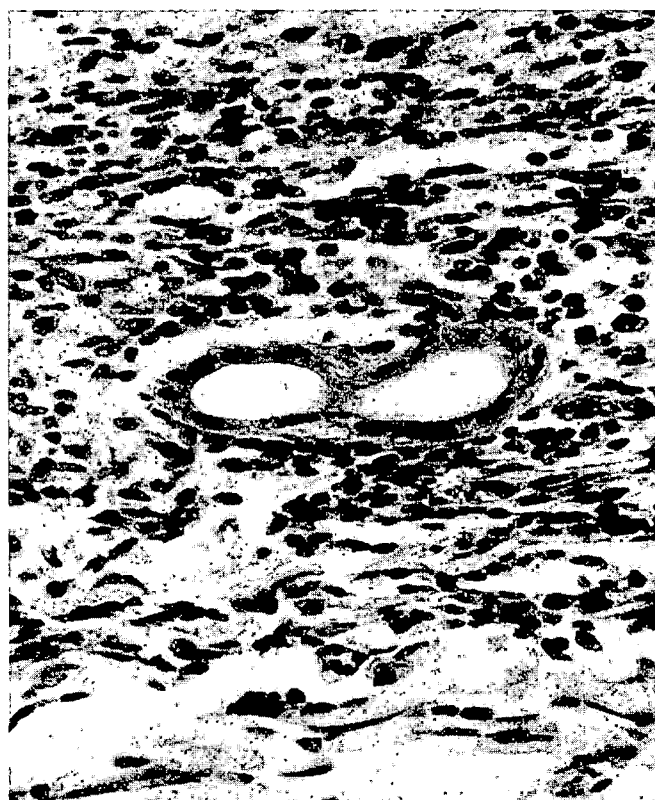


Fig. 1 Delayed hypersensitivity reaction in ear of dog sensitised with measles virus. Perivascular infiltration of small lymphocytes and some polymorphonuclear leukocytes was seen 24 h after challenge with canine distemper viral antigen prepared as previously indicated. Haematoxylin and eosin stain, magnification $\times 224$.

TABLE 2 Delayed hypersensitivity reactions in dogs

Sensitising antigen	Challenge antigen		
	Canine distemper	Measles	NL-DK-1
Canine distemper	3/3*	2/3	0/3
Measles	3/3	3/3	0/3
NL-DK-1 (control)	0/2	0/2	0/2

* Numerator indicates number of positive reaction sites, denominator indicates number of dogs tested.

The purpose in our first experiment was to determine by comparison of lesions and histological sections whether sensitisation with measles virus would cause a dog to respond to a challenge with canine distemper antigen with a typical delayed hypersensitivity response (Table 1). Dogs sensitised with canine distemper virus or NL-DK-1 cells served as controls. Fourteen days later dogs were inoculated bilaterally with both canine distemper antigen and the NL-DK-1 cell antigen as control. After 24 h, under general anaesthesia, punch biopsies approximately 20 mm in diameter were removed at the sites of inoculation from one ear. After 48 h, similar biopsies were made on the other ear. Tissues were fixed in 10% neutralised formalin and stained with haematoxylin and eosin. The cell types seen in the lesions after 24 h were a mixture of mononuclear cells and polymorphonuclear leukocytes (Fig. 1). By 48 h the former were predominant. This response is similar to that reported for other antigens that induce delayed type hypersensitivity reactions in the dog¹⁰.

In the second series of experiments, we extended the observations of delayed hypersensitivity responses to both homologous and heterologous antigens. One month after the first inoculation, test inoculations were made bilaterally on each ear with all three antigens. Table 2 shows that animals sensitised with canine distemper virus exhibited delayed hypersensitivity reactions when challenged with canine distemper or measles antigen. Animals sensitised with measles virus and challenged with both antigens also exhibited delayed hypersensitivity reactions. Sensitisation and challenge with preparations of NL-DK-1, which were uniformly negative, served as controls to assure that the reactions seen were not a result of response to components of the culture system. It is possible that common envelope antigens acquired from the membrane of the NL-DK-1 cells could be involved in cross reactions. This would be true if the acquired antigens were masked or inactive on the NL-DK-1 cells but were exposed and active when incorporated in the viral envelope.

So far no underlying immunological mechanism has been identified for heterotypic immunity, which is the veterinary term for the concept of using one virus to provide protection against another. This demonstration of delayed hypersensitivity to measles and canine distemper virus, and of cross reactions suggest that cell-mediated immune response is the mechanism by which measles vaccine protects pups against canine distemper virus.

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Natural resistance to *Salmonella* infection, delayed hypersensitivity and Ir genes in different strains of mice

THE development of immunity to an intracellular bacterial infection such as that produced by *Salmonella typhimurium* in mice can be followed by the fall in the numbers of living bacteria in the liver and spleen. Resistance to infection appears at about the same time as delayed hypersensitivity to appropriate antigens of the infecting organisms and both may be taken as indicators of a cell-mediated immune response¹. Inbred mouse strains differ widely in their resistance to salmonella infections and one possible explanation is that they differ in their ability to produce cell-mediated immune responses in general. This has been tested by comparing the degrees of delayed hypersensitivity detectable in different strains of mice sensitised with a number of unrelated antigens. The results discussed here disprove the hypothesis but suggest a more interesting one.

Six strains of inbred mice of differing ancestry² were taken and the LD₅₀ for each, calculated after subcutaneous infection with *S. typhimurium* C5 (Fig. 1). The mouse strains could easily be separated into those sensitive to less than 100 organisms and those resistant to over 10⁵. No intermediate grades of resistance were found.

An extract was prepared from a 48 h culture of *S. typhimurium* by an adaptation of the method of Collins and Mackaness³ and the final protein concentration adjusted to 1 mg ml⁻¹. Mice infected with *S. typhimurium* 8 d previously were challenged by the injection of 0.025 ml of extract into the right hind footpad. The left hind footpad was injected with phosphate-buffered saline, and similar injections were made in unsensitised mice. Other mice of each strain were sensitised with BCG, picryl chloride and oxazolone. Mice

Mouse strain	LD ₅₀
Balb/c	<10
C57Bl	2.10
B10.D2(new line)	5.10
DBA/2	2.10 ⁵
C3H/He	1.10 ⁶
A/JAX	2.10 ⁶
CBA	1.10 ⁷

FIG. 1 LD₅₀ of inbred mouse strains after subcutaneous injection of *Salmonella typhimurium* strain C5.

TABLE 1 Delayed hypersensitivity reactions of the different mouse strains to all the sensitising agents

Sensitising agent	Balb/c	Sensitive strains		Increases*	DBA/2	Resistant strains			Increases*
		C 57Bl	B10.D2			C3H	A/JAX	CBA	
<i>S. typhimurium</i>									
× 10 sens. × 10 ³ res. C5	0.08	0.03	0.12	0.08	0.18	0.20	0.29	0.24	0.23†
× 10 sens. × 10 res. C5	0.08	0.03	0.12	0.08	0.14	0.13	0.12	0.26	0.16†
× 10 ⁴ sens. × 10 ⁴ res. M525	0.09	0.25	0.12	0.15	—	0.31	0.27	0.44	0.34†
Picryl chloride	0.08	0.05	0.05	0.06	0.05	0.06	0.11	0.09	0.08§
Oxazolone	0.07	0.11	0.18	0.12	0.12	0.09	0.11	0.12	0.11§
BCG	0.13	0.36	0.27	0.25	—	0.18	0.12	0.32	0.21§

* Increased thickness in mm of footpads (*S. typhimurium* and BCG experiments) or ears (picryl chloride and oxazolone experiments). Corrected means of 24 h and 48 h values. Comparing resistant and sensitive strains, † $P < 0.01$; ‡, $P < 0.05$; §, not significant.

were sensitised to BCG by intravenous injection of 10^5 live organisms and challenged 5 weeks later with 0.025 ml of tuberculin purified protein derivative (PPD) ($10 \mu\text{g ml}^{-1}$). Picryl chloride and oxazolone were applied to the clipped backs of the mice as 0.1 ml of a 3% solution in alcohol. Mice were challenged after 7 d with 2 drops of a 1% solution in olive oil spread on the right ear lobe, using pure olive oil as a control on the left.

Increases in thickness of footpads and ears were measured by a micrometer 4, 24 and 48 h after challenge. The immediate reaction subsided at 6 h and was followed by an increased reaction reaching a maximum at 24–48 h. This was accompanied by a mononuclear cell infiltration into the area, typical of a delayed hypersensitivity reaction. All the results have been corrected for the increases found in sensitised and unsensitised controls.

In the first series of experiments the sensitive strains were sensitised with ten *S. typhimurium* organisms and the resistant with 10^3 . Although the resistant strains clearly developed an increased delayed hypersensitivity reaction to the extract, this could conceivably have been a result of the larger sensitising dose they had been given. All the mouse strains were therefore sensitised with a low dose of *S. typhimurium*. The resistant strains still gave a significantly greater reaction, which was confirmed by a similar experiment in which all strains of mice were given a high sensitising dose of the avirulent strain of *S. typhimurium*, M525, (Fig. 2a). In contrast, the responses to BCG, picryl chloride and oxazolone (Fig. 2b) were similar in all strains of mice (Table 1). Strains of mice naturally resistant to *S. typhimurium* infection therefore show a greater capacity than sensitive strains to develop delayed hypersensitivity reactions to *S. typhimurium*. This is, however, a specific reaction to a salmonella antigen or antigens and not a general capacity to develop good cell-mediated immunity. This finding is in agreement with that of Robson and Vas³, who were able to immunise their resistant more readily than their susceptible mouse strains, using a variety of salmonella antigens. They suggested that the immunity produced was cell mediated and pointed out that the response to other organisms such as *Listeria monocytogens* and *Cryptococcus neoformans* did not run parallel.

The differences in resistance to salmonella infection of the inbred strains of mice must clearly have a genetic basis. The polarisation into strains of high and of low resistance with the absence of intermediate strains suggests that only a small number of genes, or even only one, is involved. Here our results differ from those of Robson and Vas who found that C3H/HeJ strains were susceptible and DBA/2J were intermediate. Both they and we used *S. typhimurium* C5 (obtained from Professor Rowley) as the challenge organism. The difference in results may well be due to the different routes of infection used, intraperitoneal by Robson and Vas, subcutaneous in our present work. Mice infected intraperitoneally are frequently more susceptible than when infected by the subcutaneous or intravenous routes. In the

poorly protected peritoneal cavity, bacteria have a chance to grow up; effectively increasing the inoculum^{4,5}. This is supported by the relatively low doses of bacteria which killed A/J mice in Robson and Vas's experiments compared with ours. Precise comparison of results is made difficult because we have expressed resistance in terms of LD₅₀ whereas Robson and Vas have measured prolongation of survival time.

Comparison of the results presented here with those of Levine⁶ and of Vaz *et al.*⁷ shows that the resistant strains A, CBA and C3H are those which readily produced antibodies to low doses of a benzylpenicilloyl-bovine gamma globulin complex (BPO-BGG) or of ovomucoid but gave little or no response to ovalbumin. In contrast the susceptible strains C57Bl, Balb/c and B10-D2 responded poorly to BPO-BGG and ovomucoid but well to ovalbumin. DBA/2 mice which were the least resistant of the resistant group are anomalous in that although they responded to the salmonella extract they did not respond to BPO-BGG or to ovomucoid and relatively poorly to ovalbumin. Moreover, when sensitised with large

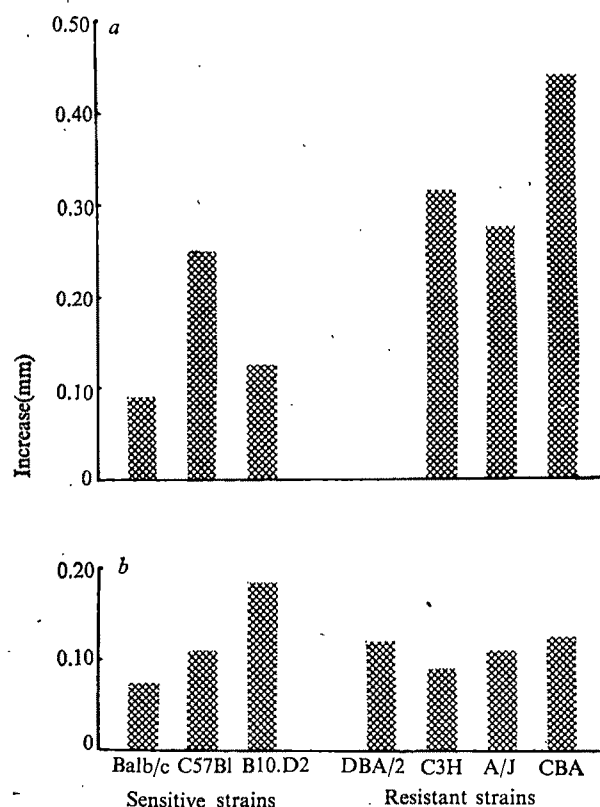


FIG. 2 a, Delayed hypersensitivity reactions to salmonella extract in mouse strains sensitised with 10^4 *S. typhimurium* strain M525. b, Delayed hypersensitivity to oxazolone. Increased thicknesses in mm of footpads (a) and ears (b) are the corrected means of the 24 h and 48 h values.

doses of the avirulent strain of *S. typhimurium* M525 they invariably died within 4 h of being challenged with the salmonella extract, apparently with some acute hypersensitivity reaction. Delayed hypersensitivity could not therefore be measured in these experiments.

Production of antibody to ovomucoid, ovalbumin and BPO-BGG in mice is thought to be controlled by immune response (Ir) genes⁶⁻⁹ and these are closely, though perhaps not invariably, associated with histocompatibility genes. In the data so far, the resistant strains A/J are H2a, CBA and C3H are H2k, of the susceptible strains, C57Bl are H2b and Balb/c H2d. DBA/2 is again odd in being resistant, but H2d. Ir genes are generally dominant. Preliminary experiments with the F₁ generation of a Balb/c, CBA cross show that resistance is also dominant. Further experiments with backcrosses to check the relations between resistance and antibody responses to ovomucoid and ovalbumin and H2 types are in progress. At present, we suggest that a major factor in the resistance of mice to salmonella is the presence of an Ir gene controlling the response to some unknown but probably protein antigen present in *S. typhimurium*. Further characterisation of this antigen is required. In the extensive literature^{10,11} on the basis of genetic resistance to salmonella infections in mice, many effector mechanisms such as antibody production, cellular immunity, phagocytic activity and macrophage bactericidal capacity have been considered. Current work on Ir genes using synthetic and other antigens may help solve the problems.

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Specific degranulation of human polymorphonuclear leukocytes

THE human polymorphonuclear leukocyte (PMN) contains two types of cytoplasmic granules, specific or heterophil granules and azurophil granules¹. These granules contain enzymes and various non-enzymatic proteins. The specific granule contains alkaline phosphatase and most of the lysozyme of the cell while the azurophil granule contains the acid hydrolases and other enzymes which identify it as a lysosome¹. Recent work² has shown that in the degranulation accompanying phagocytosis the specific or heterophil granules combine with the phagosome first, followed by the azurophil granules. This implies that degranulation may be a complex event with more than one signal involved. If this were true

then one might expect that the events should be separable, that is, that one type of granule might be stimulated and the other little affected. Experiments in our laboratories have indicated that only one type of PMN granule responds to phorbol myristate acetate (PMA). Five minutes after application of nanogram quantities of the agent the cell begins to show large intracellular vacuoles. Granules which demonstrate an alkaline phosphatase reaction disappear in 30 min while those staining for myeloperoxidase remain³. Here we report that PMA causes both a dose and a time dependent release of enzyme activity associated with specific granules while those associated with azurophil granules or lysosomes remain associated with the cell as did a cytoplasmic marker enzyme glucose-6-phosphate dehydrogenase.

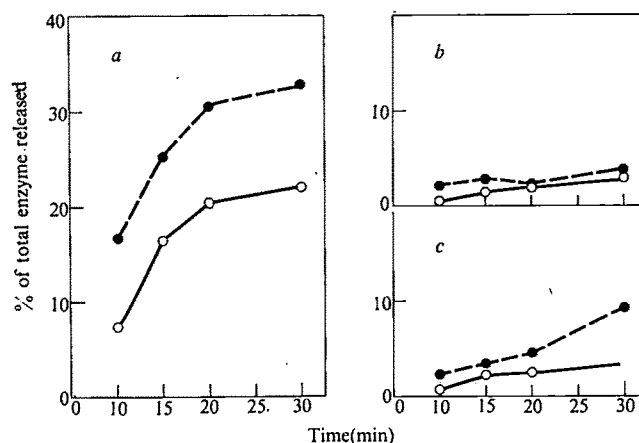


Fig. 1 a, Lysozyme; b, myeloperoxidase; c, β -glucuronidase. PMN (3×10^7) were incubated with or without PMA added at 0 time. The percentage of total enzyme released into the HBSS solution is indicated and in each case a time-matched control without PMA was subtracted from the total enzyme released. o, 5 ng ml⁻¹ PMA; •, 20 ng ml⁻¹ PMA.

The human PMN used in these studies were all sedimented through dextran and washed as previously described⁴. Red blood cells were lysed by 0.87% ammonium chloride⁵. The cells in these preparations were approximately 85% PMN, the remainder comprising lymphocytes, monocytes and platelets. These cells were incubated in a CO₂ incubator in Hanks Balanced Salt Solution (HBSS) without phenol red or serum. At the end of each incubation the cells were sedimented at 250 g and the supernatant was used for determination of enzyme activity released from the cells. The cell pellet was resuspended in 0.2 M sucrose plus 0.1% Triton X100 and then quick frozen (acetone-dry ice) and thawed five times. The enzyme activity in this material was defined as cell associated. Addition of this activity and that released from the cells is defined as total enzyme activity. The pH of the supernatant was not altered by the addition of PMA. The addition of the supernatant to the enzyme reaction mixtures described below did not alter the pH of these mixtures. The procedures for assay of lysozyme, myeloperoxidase, glucose-6-phosphate dehydrogenase⁶, β -glucuronidase⁷ and alkaline phosphatase⁸ are described elsewhere. In each experiment, illustrated in Table 1 and Fig. 1, assay of cell-associated enzymes in the presence and in the absence of PMA indicated that PMA did not inhibit or enhance the activity of any of the enzymes tested. The cells were not permeable to trypan blue either at the start or end of each experiment. In addition, the cells did not lyse during the incubation, since they fail to release either the cytoplasmic enzyme (glucose-6-phosphate dehydrogenase; not shown) or enzymes of the azurophil granule.

TABLE 1 Effect of PMA on the release of enzymes from polymorphonuclear leukocytes

	Incubation time (min)	Lysozyme	Percentage of total enzyme released* Alkaline phosphatase	Glucuronidase
Control	15	4.7	—	7.1
5 ng ml ⁻¹ PMA	15	21.4	29.5	6.4
20 ng ml ⁻¹ PMA	15	31.5	41.0	5.7
20 ng ml ⁻¹ PMA	5	7.3	10.4	3.2

* Calculated from loss of cell-associated enzyme during incubation of PMN with PMA, compared with cell-associated enzyme at 0 time and cell-associated enzyme of matched control incubated in the absence of PMA. The presence of phosphate buffer in the medium (HBSS) precludes the determination of alkaline phosphatase activity in the medium. Alkaline phosphatase activity released in this table therefore represents the difference between total enzyme activity of control and experimental values.

PMA was obtained from Consolidated Midland Corp. Brewster New York. Each lot was examined by a mass spectrometer (showed a single molecular weight of 616) and by thin layer chromatography (migrated as a single spot).

Table 1 and Fig. 1 illustrate the effects of PMA on the release of enzyme from the PMN. Table 1 shows that both lysozyme and alkaline phosphatase responded similarly to comparable concentrations of PMA. In this experiment the amount of β -glucuronidase released was similar in control cells and cells exposed to PMA. Figure 1 shows the time course of release of lysozyme, myeloperoxidase and β -glucuronidase after treatment with 5 and 20 ng ml⁻¹ PMA. In this experiment all enzyme activity shows an increase which is dependent on both PMA concentration and time of exposure. However the enzymatic activity represented in the specific granule (lysozyme) rises sharply and early. The lines in the graph are not carried through the origin since little or no release of enzyme activity is detectable in the 5 min after addition of PMA (see Table 1). In all of these experiments the cytoplasmic enzyme glucose-6-phosphate dehydrogenase did not appear in the external media.

Our data indicate that PMA causes the external degranulation of the PMN specific granule. Enzyme activity associated with this organelle is either present in the media and (or) lost from the cells after exposure to PMA. This event depends both on dose and time. Enzyme activities associated with azurophil granules remain almost entirely associated with the cell. These findings are consistent with observations that the alkaline phosphatase reaction is absent in cells exposed to PMA while large myeloperoxidase-negative vacuoles arise within the cells³. In addition myeloperoxidase-staining organelles continue to be present in cells containing many vacuoles³. This is the first report of external degranulation of specific granules alone. One of the early effects of PMA is to cause a rapid rise in intracellular cyclic GMP concentration in both 3T3 fibroblasts and human platelets⁹. Further, our previous experiments¹⁰ have implicated cyclic GMP as the intracellular mediator of the action of PMA on the PMN to enhance chemotaxis. Other workers have also indicated that external degranulation of PMN which involves both specific and azurophil granules¹¹ may be enhanced by agents which increase intracellular cyclic GMP and by cyclic GMP itself^{12,13}. This suggests that PMA causes external degranulation by a mechanism which may be initiated or modulated by an increase in cyclic GMP concentration. As Bainton² has demonstrated, however, internal degranulation involves both types of granular organelles in sequence; first the specific granule and then the azurophil granule rapidly fuse with the phagosome. A similar sequential mechanism probably also operates in external degranulation. If this is the case, then exposure to PMA results in the initial half of the event, fusion of the specific granule with the external cell membrane. Subsequent events leading to fusion of azurophil granules with the membrane do not occur. Our observations, then, combined with those of Bainton² suggest that two signals may exist for degranulation.

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Calcium does not uncouple oxidative phosphorylation in tightly-coupled mitochondria from Ehrlich ascites tumour cells

ENERGY-linked Ca²⁺ accumulation occurs in mitochondria isolated from different tissues and species (for reviews, see refs. 1 and 2). If the Ca²⁺ concentrations are kept relatively low, such accumulation does not lead to any apparent biochemical damage to the mitochondria, particularly to those processes associated with ATP synthesis. Once the concentration of Ca²⁺ in the incubation system approaches values of 100–150 nmol per mg protein, however, the ion induces mitochondrial swelling, release of the accumulated Ca²⁺ and total loss of acceptor control of respiration^{3–6}. The latter situation is brought about through an increase in state IV respiration to a rate which approaches that of state III.

Other events which occur include stimulation of ATPase activity, greatly diminished ADP-ATP exchange and ^{32}P -ATP exchange activities and P/O ratios^{4,6}. All these events reach maximum at approximately identical concentrations of Ca^{2+} and so seem to reflect some common interaction of this ion at the intact (inner) membrane with which these energy-linked reactions are intimately associated.

On studying the interaction of Ca^{2+} with tightly coupled mitochondria isolated from Ehrlich ascites tumour cells (refs 7, 8 and R. F. W. T. and F. L. B., submitted for publication) these organelles showed membrane-linked responses to Ca^{2+} quite uncharacteristic of those seen in mitochondria isolated from most normal mammalian tissues, for example rat liver. The most conspicuous uncharacteristic response in these tumour mitochondria was the inability of Ca^{2+} to uncouple phosphorylation from respiration. The mitochondria are highly tolerant to exceptionally high concentrations of Ca^{2+} . Consequently, membrane-bound, energy-linked reactions normally grossly modified in the presence of 100–150 nmol Ca^{2+} per mg protein are either insensitive to or only slightly modified by concentrations as high as 300 nmol per mg protein. This finding suggests an altered metabolic behaviour in some tumour cells.

Mitochondria were isolated from a hyperdiploid strain of Ehrlich ascites tumour cells, by methods described elsewhere^{7,8}. EDTA was present in the original isolation medium but was omitted from the medium used for the final washings of the mitochondria. The acceptor control of respiration and oxidative phosphorylation were both measured as described in the legends accompanying the appropriate figures. Oxygen uptake was measured using an oxygen electrode coupled to a Rikadenki pen recorder.

ADP-ATP and ^{32}P -ATP exchange activities were measured as previously described⁹, with the exception that in the case of the ADP-ATP exchange reaction the adenine nucleotides were separated by DEAE-cellulose paper chromatography¹⁰. ATPase activity was monitored by measuring the appearance of P_i in the medium using the method of Baginski *et al.*¹¹

Sonication of the mitochondria was carried out using six 15-s intermittent exposures on an MSE 100 W sonicator, using a small probe. The suspension of mitochondria was maintained on ice during this period. The acceptor control ratio after sonication was one.

Ca^{2+} accumulation by mitochondria was measured using $^{45}\text{Ca}^{2+}$; Millipore filters (pore size 0.45 μm) were used to separate the mitochondria from the suspending medium.

The reagents used were all of AR grade. Adenine nucleotides were obtained from C. F. Boehringer and Sonne, Mannheim, Germany. Radioactive $^{45}\text{Ca}^{2+}$ was obtained from the Radiochemical Centre, Amersham, England. ^{32}P was obtained from Lucas Heights, New South Wales, Australia.

The traces shown in the inset to Fig. 1 illustrate the influence of high calcium concentration on the rate of respiration before (state IV) and after (state III) addition of ADP. Trace *a* indicates that ADP stimulated respiration some 3.5-fold in the absence of added Ca^{2+} . The amount of ADP added was such that state III respiration proceeded to anaerobiosis.

Trace *b* was obtained with an identical incubation medium except that Ca^{2+} (660 nmol) was added to the system before the addition of mitochondria. With the particular sequence of additions made in these experiments (Ca^{2+} before mitochondria) it is not possible to measure quantitatively the amount of extra oxygen taken up by the mitochondria due to the Ca^{2+} present in the medium. The rate of oxygen uptake was, however, qualitatively greater for a short period immediately following the addition of mitochondria; this increased rate would reflect the stimulation by Ca^{2+} of state IV respiration in these mitochondria as reported elsewhere (ref. 7, and submitted for publication). A most unusual feature of the trace is that despite accumulation and retention of this large

amount of Ca^{2+} by the mitochondria (submitted for publication) (and see below), the rate of respiration after accumulation was complete and did not increase significantly as it does with rat liver mitochondria^{1,4,7}. Also addition of ADP had no effect on the rate of respiration. These experiments are described in more detail elsewhere (submitted for publication).

The remaining data in Fig. 1 were obtained from traces similar to those described above but over a wide range of Ca^{2+} concentrations. The ADP-stimulated rate of respiration gradually declines to approach the same as that in the absence of ADP at about 250 nmol per mg protein. The acceptor control ratio which is maximal in the absence of added Ca^{2+} also gradually declines to a value approaching 1 at the same high level of Ca^{2+} .

Three features of these data thus contrast with the events that are seen under identical experimental conditions with mammalian mitochondria such as those isolated from rat liver (compare with data from ref. 4): (a) the ability to accumulate and retain high levels of Ca^{2+} , (b) the complete lack of an increase in the resting rate of state IV respiration once the high levels of Ca^{2+} have been accumulated by the mitochondria and (c) the inhibition of succinate-supported state III respiration.

Although the acceptor control ratios obtained with both rat liver and the ascites tumour mitochondria each approach a value of unity as the concentration of Ca^{2+} in the medium is increased (contrast data in ref. 4), it is important to note that each does so for entirely different reasons. With the liver mitochondria the value approaches unity because the rate of state IV respiration increases to that of state III.

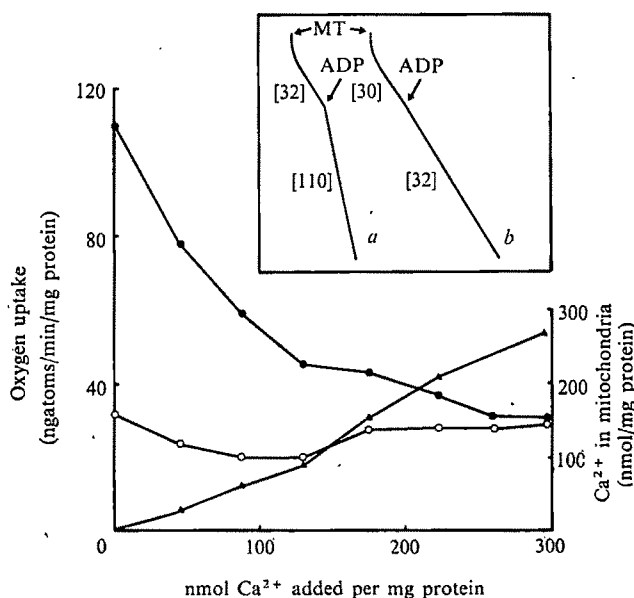


Fig. 1 Influence of Ca^{2+} concentrations on states III and IV of respiration in intact mitochondria isolated from Ehrlich ascites tumour cells. The incubation system contained (in order of addition) 80 mM sucrose, 16 mM Tris-HCl (pH 7.4), 1 mM (K) phosphate, 7 mM succinate, increasing concentrations of Ca^{2+} (containing $^{45}\text{Ca}^{2+}$) as indicated and 2.2 mg of mitochondrial protein. The total volume was 1.5 ml and the reaction temperature 25° C. Oxygen uptake in state IV (○) was measured for approximately 2 min before the addition of 1 mM ADP. When the state III trace (●) approached anaerobiosis (usually approximately 4 min after addition of ADP) a sample of medium was removed and filtered (Millipore; pore size, 0.45 μm) and the mitochondria analysed for the presence of $^{45}\text{Ca}^{2+}$ (▲). Numbers in brackets in the inset are the rates of O_2 uptake in the absence (a) and presence (b) of 660 nmol Ca^{2+} .

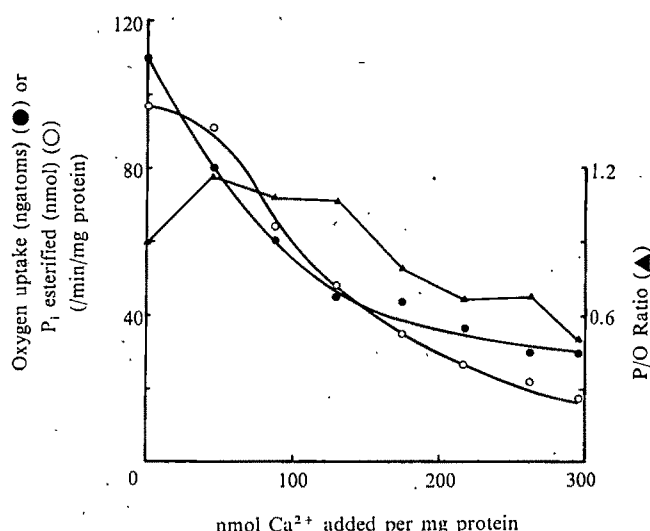


Fig. 2 Influence of Ca^{2+} concentration on the P/O ratio in intact mitochondria from Ehrlich ascites tumour cells. The incubation system was identical to that described in Fig. 1 except that $^{45}\text{Ca}^{2+}$ was not added. Also, phosphate (containing approximately 0.5×10^6 c.p.m. $^{32}\text{P}_i$) was added with the ADP. An aliquot of the reaction medium was removed into perchloric acid for assay of esterified $^{32}\text{P}_i$ at a known time, approximately 3 min, after addition of the ADP plus $^{32}\text{P}_i$. The P/O ratios were calculated from the total oxygen uptake from the time of the combined addition of ADP and $^{32}\text{P}_i$ until the reaction was stopped in perchloric acid.

With the tumour mitochondria, however, state III respiration declines to approach that of state IV.

The unusual respiratory behaviour of the tumour mitochondria induced by Ca^{2+} as described above prompted an examination of the effect of this ion on the P/O ratio. Data from one such experiment are shown in Fig. 2. The curve relating phosphate esterification to Ca^{2+} concentration shows only a gradual decline over the entire range of Ca^{2+} concentrations examined. At no stage is there any sign of the marked response of this function to Ca^{2+} as seen for example in rat liver mitochondria⁴. A most unusual and potentially significant consequence of the data is that since both state III respiration and phosphate esterification decline to similar extents there is little significant change in the P/O ratio. Thus at a Ca^{2+} concentration of 300 nmol per mg protein, the P/O ratio for succinate is still only about half of that observed in the absence of Ca^{2+} .

Since the partial reactions mentioned in the first few paragraphs reflect aspects of the mechanisms of oxidative phosphorylation, we examined the effect of Ca^{2+} on these reactions. Figure 3 shows the influence of Ca^{2+} on the $^{32}\text{P}_i$ -ATP exchange activity in intact and sonic ascites mitochondria. Since the data in the literature for the rat liver system is inadequate for the present purposes, experiments carried out with mitochondria from this tissue have also been presented. The gradual decline in activity in the ascites mitochondria is again clearly evident and contrasts with the sharp response seen with rat liver mitochondria at about 100 nmol per mg protein. The response to Ca^{2+} of this exchange in intact ascites mitochondria is almost identical to that observed with sonic preparations suggesting that Ca^{2+} does not interact with the intact ascites mitochondria in the same way as it does with the intact rat liver mitochondria.

The response of the ATPase and ADP-ATP exchange activities to Ca^{2+} in intact and sonic preparations is shown in Fig. 4. The pattern of ATPase activity seen here with intact mitochondria most likely reflects that due to accumulation of the Ca^{2+} (see refs 5 and 8) and not that of the 'irreversible' ATPase seen in other mitochondrial preparations⁵. In

the present incubation conditions, sonic particles are unable to accumulate Ca^{2+} and this is reflected in the low ATPase activity seen with these preparations. Also in complete contrast with rat liver mitochondria⁴, the ADP-ATP exchange activity in intact ascites mitochondria is completely unaffected by all Ca^{2+} levels up to 350 nmol per mg protein.

A comparison of the present body of data (refs 7, 8 and submitted for publication) with that presented previously⁴⁻⁶ reveals that mitochondria from Ehrlich ascites tumour cells exhibit a response to Ca^{2+} which is distinctly different from that exhibited by all other mammalian mitochondria so far examined. A number of energy-linked reactions intimately linked to the process of oxidative phosphorylation change completely and irreversibly when mitochondria isolated from most normal mammalian tissue are exposed to excessive Ca^{2+} levels. Such changes are either absent or considerably dampened when tightly coupled mitochondria from Ehrlich ascites tumour cells are exposed to similar high levels of Ca^{2+} , which indicates that these mitochondria are atypical in their ability to tolerate extremely high concentrations of Ca^{2+} . Mitochondria from L1210 mouse ascites tumour cells have also been shown to tolerate high levels of Ca^{2+} (ref 12).

Important evidence reflecting this tolerance is shown in Fig. 1 where a complete lack of increase in the resting rate of state IV respiration was observed even after the accumulation of Ca^{2+} concentrations as high as 280 nmol per mg protein. In reactions such as the $^{32}\text{P}_i$ -ATP exchange where some change did occur, it was usually gradual and in no way reflected the marked change which occurs at Ca^{2+} levels of about 100–150 nmol per mg protein with, for example, rat liver mitochondria. Indeed, in the ascites preparations the response of the exchange to Ca^{2+} was practically identical in intact and sonic preparations.

The molecular events that occur when Ca^{2+} interacts with normal mammalian mitochondria to induce the (irreversible) modifications to the above energy-linked reactions are unclear. All the reactions are membrane-linked and show maximal sensitivity to Ca^{2+} when the mitochondria are most intact as revealed by the tightness of coupling of phosphorylation

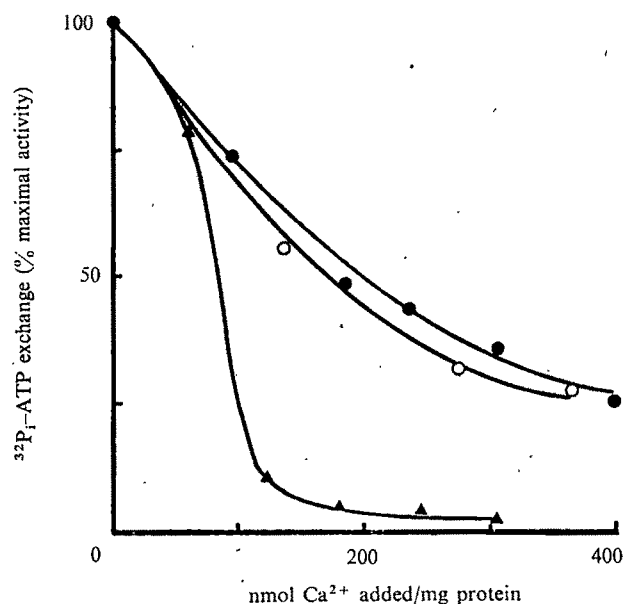


Fig. 3 Influence of Ca^{2+} concentration on the $^{32}\text{P}_i$ -ATP exchange activity in intact and sonic mitochondria isolated from Ehrlich ascites tumour cells. The experimental conditions were exactly as described by Bygrave and Lehninger⁸. ●, Intact ascites mitochondria (43); ○, sonic ascites mitochondria (11); ▲, intact liver mitochondria (88). Values in parentheses indicate nmol $^{32}\text{P}_i$ exchange per min per mg mitochondrial protein in absence of added Ca^{2+} .

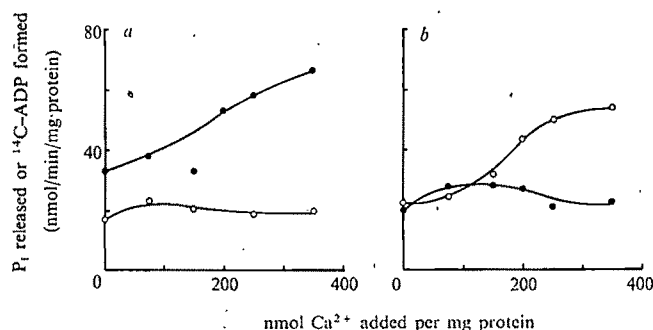


FIG. 4 Influence of Ca^{2+} concentration on ATPase (a) and ADP-ATP exchange (b) activities in intact and sonic mitochondria from Ehrlich ascites tumour cells. The experimental conditions were exactly as described by Bygrave and Lehninger⁹. ●, Intact mitochondria; ○, sonic mitochondria.

to respiration. Despite the uncertainties of the molecular events involved in uncoupling by Ca^{2+} it is quite clear that the Ca^{2+} -induced mechanism operating in normal mitochondria either does not exist or is grossly modified in mitochondria isolated from Ehrlich ascites tumour cells.

A second significant feature of our data is that the tumour mitochondria are not only tolerant to high levels of Ca^{2+} but also no longer respond to ADP with a respiratory jump. Thus phosphorylating respiration is prevented when these mitochondria are loaded with even small amounts of Ca^{2+} , a feature not seen in normal mitochondria (see ref. 1). This property is being studied in detail in our laboratory and most likely reflects an inhibition of adenine nucleotide translocation by Ca^{2+} in these tumour mitochondria. This is discussed elsewhere (submitted for publication).

Elsewhere we have advocated that Ca^{2+} movements between the cytoplasm and mitochondria form the basis of a means of controlling Ca^{2+} -sensitive cellular metabolic processes located in one or other of these two compartments¹³⁻¹⁸. Thus the unusual interactions of the Ehrlich ascites cell mitochondria with Ca^{2+} described here is of great interest. Although it is difficult to extrapolate information from *in vitro* experiments to *in vivo* events, the intriguing possibility exists that because mitochondria from Ehrlich ascites tumour cells can accumulate and maintain unusually high concentrations of Ca^{2+} without any apparent damage, they provide an environment in the cytoplasm consisting of a high $\text{Mg}^{2+}:\text{Ca}^{2+}$ ratio. In this way metabolic processes localised in the cytoplasm outside mitochondria such as glycolysis, protein synthesis and phospholipid synthesis, all of which are enhanced by an increase in this ratio¹⁸, proceed much faster than those occurring in other tissues, whose mitochondria are less tolerant to high concentrations of Ca^{2+} . Studies on the relation of these ideas to the growth and metabolic characteristics of various tumours are under way in this laboratory.

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Response of mouse melanoma cells to melanocyte stimulating hormone

We have recently described a mouse melanoma cell line which responds to melanocyte stimulating hormone (MSH) with dramatic increases in tyrosinase activity and melanin content, as well as changes in cellular morphology and growth characteristics¹⁻³. The increase in pigmentation following exposure to MSH is shown in Fig. 1. Cyclic AMP or dibutyryl cyclic AMP may be substituted for MSH in eliciting these responses; consistent with other work suggesting that MSH acts through cyclic AMP in the control of pigmentation in vertebrates⁴⁻⁷. In our previous studies we used non-synchronised mouse melanoma cells grown in monolayer culture. Martin *et al.*⁸ have shown that synchronised mouse HTC cells respond to corticosteroids with increased tyrosine amino transferase synthesis in the late

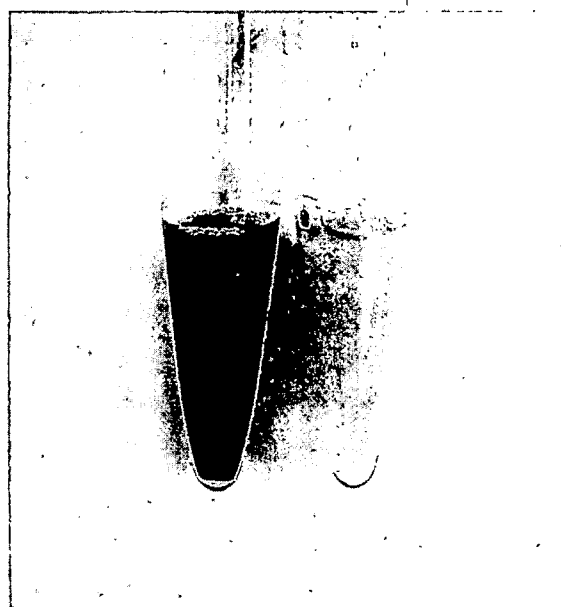


FIG. 1 Effect of MSH on pigment formation by cultured melanoma cells. 10^6 cells were inoculated into each of 4 culture flasks; 10^{-7} M MSH was added to two of the flasks. After 4 d, the cells were collected, pooled, lysed in hypotonic buffer, and photographed. MSH-treated cells are on the left.

TABLE 1 Time periods for the cell cycle of colchicine-synchronised melanoma cells

Hours after release from colchicine	Phase
0-5	Mitosis
5-15	G1
15-25	S
24-45	G2
45-	Mitosis*

* Metaphase cells were counted to determine mitosis. At 45 h, approximately 25% of the cells were in metaphase, at 48 h more than 60% were in metaphase.

G1 and early S periods of the cell cycle; similarly, Buell and Fahey⁹ have shown, using a synchronised human lymphoid cell line, that immunoglobulins G and M are expressed in late G1 and S. We wished to investigate the effects of MSH on tyrosinase activity and endogenous cyclic AMP levels in synchronised cells to determine if there is a particular hormone-sensitive phase of the melanoma cell cycle. We report here that melanoma cells responded maximally to MSH with increased tyrosinase activity and cyclic AMP content during the G2 phase.

Cloudman S91 NCTC 3960 (CCL 53) mouse melanoma cells were obtained from the American Type Culture Collection, Cell Repository and maintained in monolayer culture as previously described². For a maximal response to MSH, cells were inoculated at 5×10^5 — 10×10^5 cells per 250 ml Falcon tissue culture flask and sub-cultured weekly. Under these conditions, the cells have a doubling time of approximately 48 h. If the cells are inoculated at a higher density or sub-cultured less frequently, they tend to show increased tyrosinase activity and melanin content in the absence of MSH and the response to the hormone is less marked.

Cells were synchronised by exposure to $0.015 \mu\text{g ml}^{-1}$ colchicine for 36 h, by which time most of the cells had reached metaphase. Metaphase cells were collected by manual shaking of the culture flasks, washed three times in complete medium free of colchicine, inoculated into 100 mm Falcon tissue culture Petri dishes at 10^6 cell per dish

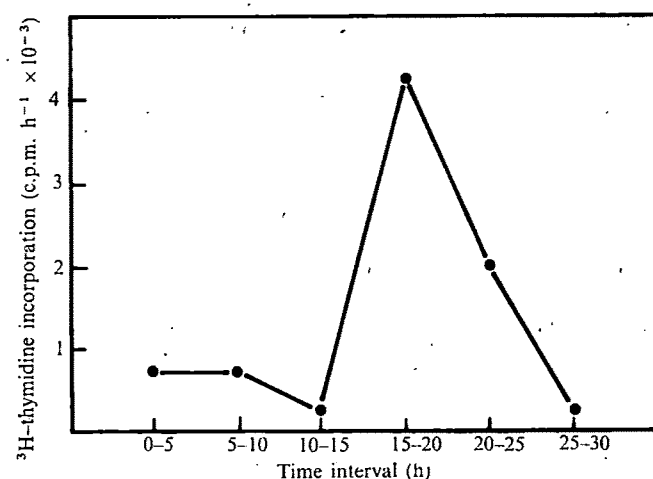


FIG. 2 Incorporation of thymidine ³H-methyl, New England Nuclear 20 Ci mmol⁻¹ by synchronised melanoma cells. Cells were incubated with $1 \mu\text{Ci ml}^{-1}$ ³H-thymidine at 5 h intervals. After 5 h in thymidine, the cells were collected, precipitated with cold 5% trichloroacetic acid, filtered on to a 0.45μ Millipore filter, and washed with 30 ml 5% trichloroacetic acid containing an excess of non-radioactive thymidine. The filters were dried in air and counted in a liquid scintillation counter. The points are averages of duplicate culture flasks.

in 10 ml medium, and incubated at 37° C in a gassed, humidified incubator. The time periods for the various phases of the cell cycle were determined by the methods of Martin *et al.*⁸ Tyrosinase activity was assayed for cells *in situ* as previously described².

Cyclic AMP was measured by modifying the method of Brown *et al.*¹⁰: cyclic AMP binding protein from calf adrenals was further purified on a DEAE-cellulose column so as to be free of phosphodiesterase activity. Theophylline was found to increase the background in the binding assay and was omitted from the reaction mix. Two different procedures were followed to extract cyclic AMP from the cells

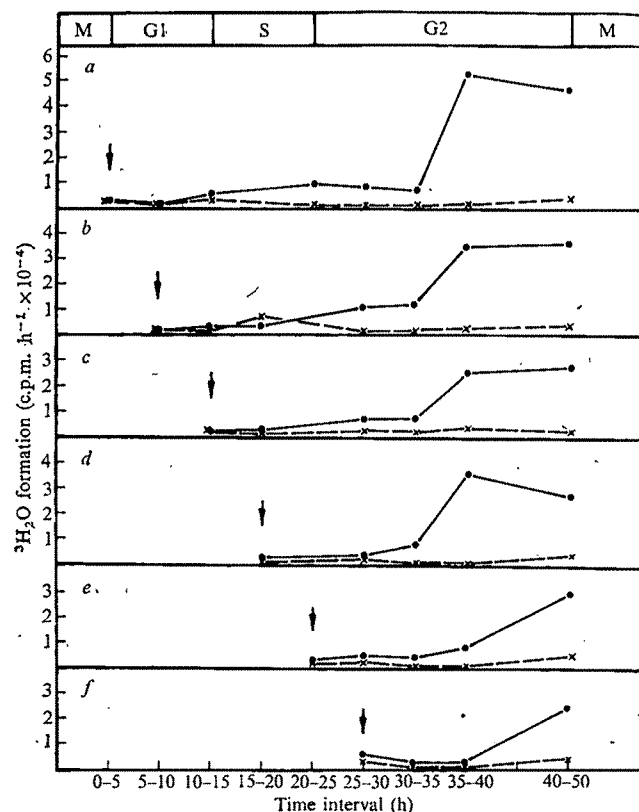


FIG. 3 Tyrosinase activity in synchronised melanoma cells. Cells were incubated with or without 10^{-7} M MSH which was added at the times indicated by arrows. 3, 5-³H-L-tyrosine (New England Nuclear 20 Ci mmol⁻¹) was added at $1 \mu\text{Ci ml}^{-1}$ to control and MSH-treated cultures at the same times that MSH was added. ³H₂O formation was measured to assay tyrosinase activity. Each point represents the ³H₂O formed h⁻¹ during the various time intervals. Zero time is when the cells were released from colchicine. The points are averages of duplicate culture flasks. X---X, Control; ●—●, MSH treated.

(see legends to Fig. 4 and Table 2) but the results were essentially the same regardless of which was used.

The time periods for the various phases of the cell cycle are shown in Table 1. The incorporation of ³H-thymidine into DNA was used to monitor the S phase (Fig. 2), and metaphase cells were counted to determine mitosis. The periods were the same whether or not MSH was added to the culture medium. MSH (10^{-7} M) was added to the cells at various times after release from the colchicine block in metaphase, and tyrosinase activity and endogenous cyclic AMP levels were followed in control and MSH-treated cells. The experiments were performed at least twice with similar results each time.

Tyrosinase activity was assayed by measuring ³H₂O released into the medium by cells incubated with 3,5, ³H-L-

TABLE 2 Cyclic AMP levels in synchronised cells following rapid extraction technique*

Time of cyclicAMP Extraction	pmol cyclicAMP per 2 × 10 ⁶ cells			
	Control Experiment	Average	MSH-treated Experiment	Average
<i>t</i> = 3 h	(1) 2.8 (2) 3.2	3.0	(1) 4.0 (2) 6.4	5.1
<i>t</i> = 12 h	(1) 2.8 (2) 4.8	3.8	(1) 3.2 (2) 4.8	4.0
<i>t</i> = 24 h	(1) 0.8 (2) 1.6	1.2	(1) 5.4 (2) 25.0	15.2
<i>t</i> = 30 h	(1) 2.0 (2) 1.6	1.8	(1) 41.0 (2) 45.0	43.0
<i>t</i> = 36 h	(1) 2.0 (2) 3.6 (3) 4.2	3.3	(1) 41.0 (2) 65.0 (3) 37.0	48.0
<i>t</i> = 48 h	(1) 2.4 (2) 3.0 (3) 3.4	2.9	(1) 25.0 (2) 45.0 (3) 35.0	35.0

* Cells were incubated as described and 10⁻⁷ M MSH was added 30 min before each sampling. Culture medium was removed by aspiration, the cells were rinsed gently with 2 ml Hanks' balanced salt solution, and ice-cold ethanol-0.2 N HCl was added to the cells with a Pasteur pipette. Removal of medium and addition of ethanol-HCl took less than 10 s. The cells were then placed at -10° C for at least 24 h and thereafter treated as described in legend to Fig. 4. The extracts were diluted in order to measure 0.2 to 5.0 pmol cyclic AMP per assay. The growth medium alone incubated in the presence or absence of MSH or with 100 pmol of added cyclic AMP contributed negligible amounts of cyclic AMP by this extraction procedure.

tyrosine. (Fig. 3a-f) Although the additions of MSH were staggered at 5 h intervals from 0-25 h, the hormone-mediated response was always maximal in the G2 phase of the cycle.

Cyclic AMP was measured at each time point following 30 min of exposure to MSH (Fig. 4 and Table 2). Although MSH increased cyclic AMP levels throughout the cell cycle, there was a dramatic rise over control values in the G2 phase of the cycle, 30-40 h after colchicine release. Control values changed very little throughout the cycle. MSH also caused an increase in cyclic AMP released to the culture medium by the cells (Table 3). The sharp increase in cyclic AMP levels in G2 seemed to precede the increase in tyrosinase activity, consistent with previous evidence that cyclic AMP is an intracellular mediator in the MSH response.

Two control experiments not shown here bear on the above results. First, when tyrosinase activity was measured in homogenates of collected cells, the results closely paralleled those obtained by measuring ³H₂O released to the medium. Thus the lag periods observed in the experiments presented here were not due to a lag in the uptake of ³H-tyrosine

TABLE 3 Cyclic AMP levels in the culture medium*

Treatment of medium†	pmol cyclic AMP per 10 ml
Without cells and without MSH	Experiment 1 < 0.1 2 < 0.1
Without cells, MSH added 30 min before sampling	Experiment 1 < 0.1 2 < 0.1
With cells and without MSH	Experiment 1 < 4 2 < 4
With cells, MSH added 30 min before sampling	Experiment 1 56 2 72

* Constituents of the culture medium were listed previously². Cells were released from colchicine and 0.25 ml medium was removed after 42 h incubation at 37° C. A sample of 2 ml ice cold ethanol-0.2 N HCl was added, and the mixture was placed at -10° C. After 24 h the mixtures were centrifuged and thereafter treated as in legend to Fig. 4. The extracts were diluted as described in legend to Table 2.

† All media were incubated for 42 h.

by the cells or to a lag in the release of ³H₂O to the medium. Second, colchicine, added again after the cells had passed through the initial mitosis, did not influence the results, providing additional evidence that the cells responded in the G2 phase.

When non-synchronised cells were treated with MSH, a rise in tyrosinase activity could be detected after a lag period of 6-9 h (ref. 3). The lag period with synchronised cells can, however, be as long as 35 h, depending when in the cell cycle MSH is added (Fig. 3). These results can be explained if it is assumed that in non-synchronised populations, the initial rise in tyrosinase activity takes place in cells that are in the G2 phase of their cycle.

Chen *et al.*⁵ have provided evidence that both MSH and cyclic AMP induce the differentiation of melanoblasts into melanocytes in organ cultures of caudal fins from xanthic goldfish. The melanoblasts are inducible shortly preceding mitosis, which may indicate that these cells are also most sensitive to MSH in the G2 phase. This result raises the possibility that vertebrate melanocytes are, in general, responsive to MSH in G2, an hypothesis which awaits further experimentation.

The relationship of the phase of the cell cycle to cellular phenotypic expression is as yet poorly understood. Varga *et al.*¹¹ have, however, recently provided evidence which explains why the melanoma cells respond to MSH only in the G2 phase. Using ¹²⁵I-labelled MSH as a tracer, they found that the cells are able to bind the hormone to their plasma membranes only in the G2 phase; there is little or no binding in G1 or S. Apparently MSH-receptors are available only in G2. Thus the MSH-mediated rise in intracellular cyclic AMP concentration is restricted to the G2 phase because this is the only time when the hormone can bind to the cells and stimulate membrane-bound adenyl

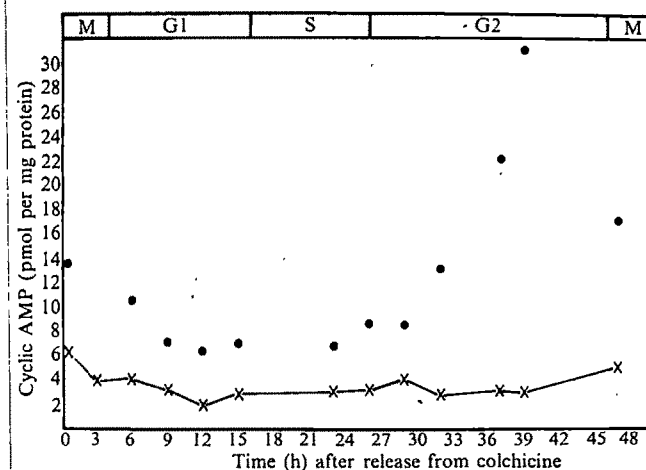


Fig. 4 Cyclic AMP levels in synchronised melanoma cells. Cells were incubated as described and 10⁻⁷ M MSH was added 30 min before each sampling. The wash procedure took 10 min and all operations were at 4° C. Growth medium was removed, cold Hanks, balanced salt solution was added, the cells were collected with a rubber policeman, washed three times in Hanks' by centrifugation, and cold ethanol containing 0.2 N HCl was added to extract cyclic AMP. After at least 24 h at -10° C the extracts were centrifuged, the supernatants removed, the pellets washed with additional ethanol-HCl, the supernatants pooled, dried *in vacuo*, and cyclic AMP measured by the method of Brown *et al.*¹⁰. Known amounts of cyclic AMP were added to aliquots of each extract as internal standards. Recovery of the standards was routinely more than 90%. The growth medium alone incubated in the presence or absence of MSH or with 100 p mol of added cyclic AMP contributed negligible amounts of cyclic AMP by this extraction procedure. The pellets were dissolved with 0.1 N NaOH and protein was estimated by the Lowry method. The points are averages of duplicate culture flasks ×-×, control; ● MSH treated.

cyclase. Presumably the increased cyclic AMP concentration then results in increased tyrosinase activity.

We do not yet know if the increase in tyrosinase activity in cells exposed to MSH is due to new synthesis of tyrosinase molecules or activation of pre-existing molecules. Localisation of the melanoma cell response to MSH in the G2 phase, however, should be helpful in the identification of the molecular intermediates involved.

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Stimulation of tumour cell dissemination by raised temperature (42°C) in rats with transplanted Yoshida tumours

HYPERTHERMIA, that is, temperatures $\geq 40^\circ\text{C}$, may have a selective destructive effect on some types of cancer cells^{1,2}, and sporadic attempts have been made to treat human cancer by raised temperatures¹⁻³. Rossi-Fanelli and colleagues have demonstrated a selective and irreversible inhibition of metabolism in a variety of tumour cells at 42°C , whereas normal cells were unaffected^{1,4,5}. These workers obtained histological regression of malignant limb tumours in patients treated by regional perfusion with prewarmed blood¹. More effective and controlled methods for inducing hyperthermia⁶, and reports that the action of cytotoxic drugs can be potentiated by hyperthermia^{7,8}, have further stimulated interest in this approach to the treatment of human malignant tumours.

Little precise information is available on optimal conditions of heating, susceptibility of various tumours, and relative effectiveness of locally applied heat compared with total body heating. Some of these problems have previously been investigated using the VX2 carcinoma in the rabbit⁹⁻¹¹, and we now report observations on the behaviour of the Yoshida tumour in response to elevated temperatures *in vitro* and *in vivo*, with particular reference to the unexpected finding of stimulation of malignant cell dissemination by inadequate heating of implanted tumours at 42°C .

In the present work, the solid form of the transplantable Yoshida tumour¹² in the rat was used. The tumour was maintained by periodic transfer of 0.1 ml (100 mg) tumour homogenate by trocar superficially into the muscle of the hind leg in outbred Wistar rats of both sexes fed an *ad libitum* diet, and weighing 200-250 g. The tumours became palpable after 6 d and increased in volume exponentially until 11 d, when the growth rate decreased in accordance with the Gompertz function for solid tumours. The animals died at 26 ± 3.1 d (260 rats) following inoculation, when the primary tumour volume was approximately 100 ml.

The sensitivity of the Yoshida cells *in vitro* to increased temperature was assessed by conventional Warburg manometry, using 3.0 ml Krebs-Ringer-phosphate buffer, pH 7.40, containing 0.013 M sodium succinate with air as the gas phase, and 0.2 ml 10% KOH in the centre well (respiration); for anaerobic glycolysis the suspending buffer was 3.2 ml Krebs-Ringer-bicarbonate-phosphate⁴, pH 7.40, containing glucose (2 g l^{-1}), with a gas phase of 95% N_2 and 5% CO_2 . At temperatures above 41°C , there was depression of both respiration and glycolysis. At 42°C , irreversible damage to the cells occurred after 4 h, as indicated by cessation of O_2 uptake and CO_2 production, failure to respire or glycolyse on transfer to fresh incubation buffer at 38.0°C , and failure to produce tumours when transplanted into rats. On the basis of these results, 42°C was selected as the most suitable temperature to use for therapy of this tumour. Above 42°C , more rapid inhibition of respiration and glycolysis occurred, but the differential heat sensitivity between cancer cells and normal cells is diminished¹³, and subject tolerance in animals and in humans dictates that the therapeutic temperature range in heated tumours is $41-42^\circ\text{C}$ ¹⁰.

Figure 1 illustrates the temperature profiles obtained when tumour-bearing rats were treated by immersion of the affected limb in a waterbath to maintain an intra-tumour temperature of 42°C for 1 h. It was not possible to achieve this without the animal's central body temperature rising to an average in excess of 41.5°C over the 60 min therapy period. The rats did not tolerate this elevated temperature; of 167 rats so treated, 151 died during heating or within

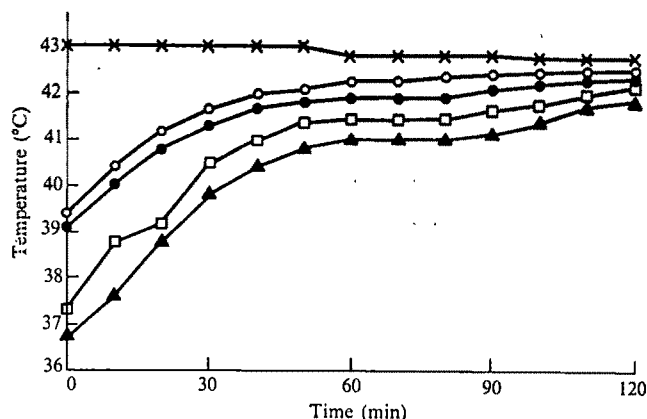


Fig. 1 Tumour and central body (core) temperature profiles for a rat treated by immersion of the tumour-bearing limb in a waterbath at 43°C . The tumours were treated on the eighth day after inoculation into the hind limb when the volume was 10.3 ± 1.3 ml, and there was no necrosis present. The temperature monitoring probes were inserted under Nembutal anaesthesia, and temperatures were measured by multi-channel direct reading electric thermometers (Model 3 GID, Light Laboratories, Brighton). Temperatures were recorded from opposite poles of the tumour (intratumour I and II), from the abdomen and rectum. The rats were heated in groups of four, and during and immediately following therapy each animal was given 1 ml 4% dextrose-0.18% NaCl solution (at 42°C) to replace fluid loss. Temperature: X, bath; O, intratumour I; ●, intratumour II; □, intra-abdominal; ▲, rectal.

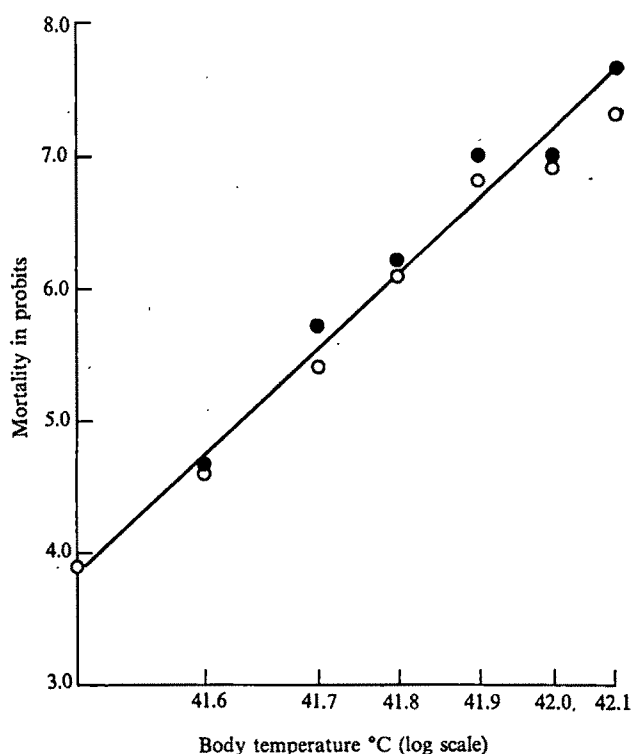


FIG. 2 Probit transformation for % mortality in a series of 200-250 g rats subjected to hyperthermia. The hind-quarters of the normal animals were placed in the water-bath, and the temperature in the muscle of the immersed limbs maintained at 42°C for one hour; the tumour-bearing rats were heated as detailed in the legend to Fig. 1. The average body temperature over the heating period was adjusted to the nearest 0.1°C. At each temperature point on the curve for normal rats there were at least eight animals with thirty animals at the 42.0°C point (total number of rats heated = 82), while for the tumour-bearing series there were at least twelve animals at each temperature point, and at 41.9°C and above there were at least thirty animals per point (total number of rats heated = 167). ○, Normal; ●, tumour-bearing rats.

the following 24 hours (survival rate for the heating = 9.6%). One rat survived for three days and nine died at 6-12 d following heating; the remaining six survivors were electively killed for pathological examination at 2-6 d after hyperthermia. As indicated in Fig. 2, the survival rate decreased rapidly when body temperatures in excess of 41.5°C were maintained for 1 h, and there was no difference in susceptibility to heat between normal rats and tumour-bearing animals. A body temperature just over 41.6°C represented the 50% lethal dose of heat, and few rats survived an intra-abdominal temperature of 42.0°C for 60 min.

There was no restraint of tumour growth and no inhibition of respiration after heating. Anaerobic glycolysis was significantly reduced immediately after heating, but returned to control values within 3 d. After hyperthermia, the tumours were oedematous and dark red in colour, and showed little microscopic evidence of cell destruction. These changes have been described previously for inadequately-heated VX2 tumours in the rabbit¹¹. In the animals that lived longer than 24 h, the mean survival time was 7.9 ± 2.5 d after hyperthermia, that is the rats died at 15.9 ± 2.5 d after transplantation, which was significantly less than the survival time in untreated animals ($P < 0.001$). Non-tumour-bearing rats that survived the hyperthermia had a normal life span.

Following implantation into the leg, the Yoshida tumour infiltrated muscle and other tissues, bone and the overlying skin. Later there was direct spread into the muscles of the

pelvis and posterior abdominal wall. From the nineteenth day after implantation, there was tumour in the retroperitoneal tissues in 38.7% of the animals (Fig. 3). In some rats the tumour had formed a large mass in the posterior abdominal wall surrounding and infiltrating both ureters. The perinephric and (or) peri-adrenal fat was infiltrated, and in advanced cases tumour had spread from the mesentery into the wall of the small intestine.

There was spread of tumour to the iliac lymph nodes by the ninth day, and later to the intra-abdominal para-aortic nodes. Tumour was observed in the upper para-aortic nodes at the level of the coeliac axis from the fourteenth day onwards in 32.4% of animals (Fig. 3). In animals with advanced tumours, the deep cervical lymph nodes adjacent to the thymus were involved, and in some, tumour had spread into surrounding fat and into the thymus.

Distant blood-borne metastases were uncommon in that pulmonary tumour emboli (Fig. 4) were observed in only two of ninety-one rats at 22 and 27 d after implantation, respectively. Involvement of the heart was commoner, and tumour was present in the myocardium in seventeen of sixty-nine rats (24.6%) with 13 to 32-d-old tumours (Fig. 3).

The liver and brain were not involved in any of the control animals.

The sixteen animals which survived heating at 42°C for 24 h or longer showed a more rapid dissemination of tumour than did unheated or sham-heated controls. This applied to direct, lymphatic, and blood-borne spread of the tumour (Fig. 3). Involvement of the retroperitoneal tissues occurred earlier, and with a significantly increased incidence, in the heated compared with the control series of animals over the same period (10-20 d following tumour implantation). Similarly, upper para-aortic lymph node involvement was observed earlier in the heated animals. Myocardial involvement was not premature, but occurred in a greater proportion of heated rats in comparison with the controls. Pulmonary involvement by tumour was observed in three of the sixteen heated rats, a much higher incidence than that in the controls. In two animals at 14 and 15 d following tumour implantation, there were multiple pulmonary emboli, and in the third, the lung parenchyma was extensively involved from the hilar aspect by mediastinal tumour which also involved the heart.

A further distinction between the control and test rats was the involvement of the portal tracts of the liver in two of the heated animals at 14 and 15 d, respectively, after tumour implantation (Fig. 5).

To exclude the possibility that manipulation of the animals and insertion of thermosensor needles into the tumour during heating might adversely affect spread of the tumour, a group of eighteen rats with 8-d tumours was heated in a bath at body temperature (38°C) for 2 h with needle probes inserted into the tumour. These sham-heated rats survived the procedure without loss and seven were killed at the fifteenth day, ten at the twenty-second day, and the remaining animal at the twenty-ninth day following tumour implantation. *Post mortem* examination of these animals showed a similar pattern of tumour spread in relation to the duration of the tumour implant as that observed in the control series (Fig. 3).

With various tumours transplanted subcutaneously in rats^{13,14} and mice^{15,16}, other workers have shown that the destructive effects of hyperthermia on susceptible tumours *in vivo* depend upon both the degree of heat and its duration. The response of the tumour can be expressed as an exponential type of curve in which approximately the same biological effect is obtained by halving the exposure time for each degree that the temperature increases above 42°C. To obtain regression in a variety of small tumours (maximum volume 2 ml) transplanted subcutaneously in rats, an exposure time of 60-90 min at 45°C is required^{13,14}.

The thermoregulatory mechanisms in rodents are less well-developed than those of man², and the animals are more sensitive to hyperpyrexia. The size of the Yoshida tumour (about 10 ml) in relation to the rat's body size implies that a considerable volume of blood (total blood volume in a

200 g rat = about 10 ml or 4.5% body weight¹⁷) is contained within the immersed tumour-bearing limb at any given instant. This precludes the maintenance of a temperature differential between tumour and normal body organs compatible with survival. A further difficulty encountered

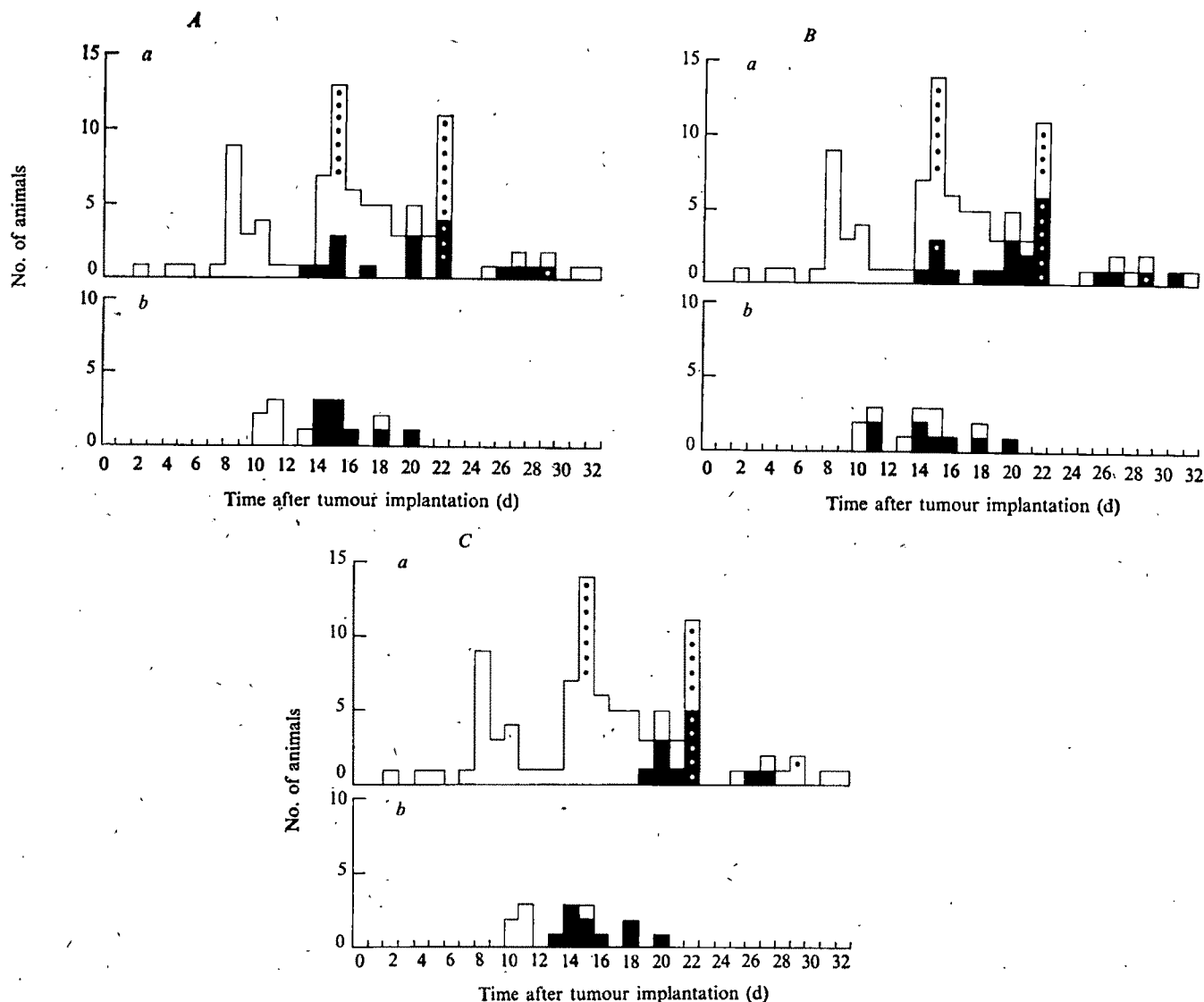


FIG 3 Incidence of tissue involvement by tumour. Examples illustrate spread by the direct, lymphatic and vascular routes. Animals were examined at post-mortem and in each of the 107 rats depicted in these frequency diagrams, the brain, abdominal and thoracic organs were examined histologically. The control and sham-heated animals were killed at intervals after implantation of the tumour to follow the time sequence of tumour spread by the various routes. All heated rats died by the twentieth day after implantation, with the exception of six animals that were electively killed to ascertain the pattern and extent of tumour spread prior to the fifteenth day. In animals that died during or within 24 h of heating, the post-mortem and histological findings did not differ from those in equivalent control rats.

In the case of the heart (A), control (a) and heated (b) animals showed myocardial involvement by tumour at the same time interval after implantation of the tumour (days 13 to 14). The incidence of heart involvement over the 10–20 d period in the heated animals was significantly higher than in the controls ($P < 0.01$). Note also that at the fifteenth day, none of the sham-heated animals showed myocardial involvement, and even at the twenty-second and twenty-ninth days, only four of eleven such animals had myocardial metastases. The incidence of myocardial involvement in the heated animals from the fourteenth to the twentieth days was nine out of ten. This suggests the increased incidence of myocardial involvement in the heated animals was not the result of mechanical factors associated with the heating procedure, such as the insertion of the needle thermistors into the tumour.

The para-aortic lymph nodes (B) at the level of the coeliac artery, and alongside the kidneys, were involved earlier in the heated animals (b) (eleventh day onwards) than in the controls (a) (fourteenth day onwards). The overall incidence over the 10–20 d period was thus higher in the heated group (50%) than in the controls (14.2%), and this difference is significant ($P < 0.02$). Insertion of the thermosensors again was without effect, and only one of seven sham-heated rats at 15 d (14.3%) had upper para-aortic lymph node involvement. In unheated and sham-heated animals with older tumours, the incidence of lymph node metastases was similar to that in heated animals at an earlier period after implantation and occurred in ten of twenty rats from the days 22–32 (50%).

The infiltration of retroperitoneal tissues (C) followed a similar pattern, with earlier involvement in the heated rats (b) at the thirteenth day compared with the nineteenth day in the controls (a), and with a significantly higher incidence in the 10–20 d period in the heated animals (62.5% compared with 7.7%, $P < 0.001$). In the heated rats showing this involvement, a large tumour mass was also present on the posterior abdominal wall. The tumour usually extended up to the lower pole of the kidneys, and often invaded the ureters. Again the sham-heated animals behaved as the other controls.

□, No tumour involvement; ■, tumour present; ●, sham-heated controls.

by previous workers has been inhomogeneous heating of tumours^{13,14}. In our work, a temperature difference of up to 0.5° C was detected by electrodes simultaneously inserted into the four quadrants of the same tumour during heating. The superficial temperature of the tumour was usually higher than the deep temperature. Two needles inserted into opposite poles to a depth of 1 cm gave a reliable guide to the temperature in the centre of the neoplasm. The depression of Yoshida tumour metabolism at 42° C *in vivo* was reversible, and together with the absence of histological damage and subsequent unrestrained increase in tumour volume, indicates that the hyperthermia was inadequate for tumour destruction.

The advanced stage of the disease in the rats that survived hyperthermia indicates that the heating accelerated dissemination of this tumour. The precise mechanisms involved are undetermined. Metastasis is a complex phenomenon comprising a sequence of at least five processes^{18,19}. The hyperdynamic state of the circulation at elevated temperature, and the local active hyperaemia, may predispose to increased 'showering' of tumour cells into the blood from the primary mass²⁰. It is now recognised, however, that there is no definite correlation between the presence of cancer cells in the blood *per se* and the development of metastases^{18,19}. Local hyperthermia may have changed the mobility of the cells of the primary tumour. Mondovi *et al.*²¹ recently described an increased antigenicity in Ehrlich ascites cells heated at 42.5° C *in vitro* for 1–3 h, and postulated that short exposure to raised temperatures caused some modification of the cell surface. The rise of central body temperature to more than 41.5° C may have altered the metabolic or biological properties of the tissues concerned

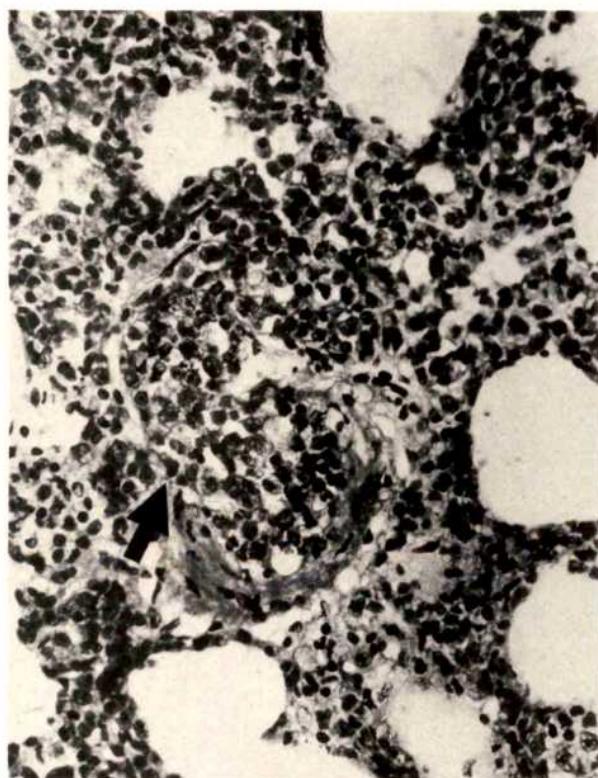


FIG. 4 Pulmonary tumour embolism in control rat killed 22 d after implantation of tumour into the leg. Note tumour cells occluding the lumen of a pulmonary vessel and spreading into the alveolar wall capillaries (arrow). This phenomenon was observed in only two rats in the large control series (ninety-one animals) at 22 and 27 d after tumour transplantation, respectively, but occurred in two of the sixteen heated rats dying at 14 and 15 d after implantation, respectively. Haematoxylin and eosin $\times 200$.

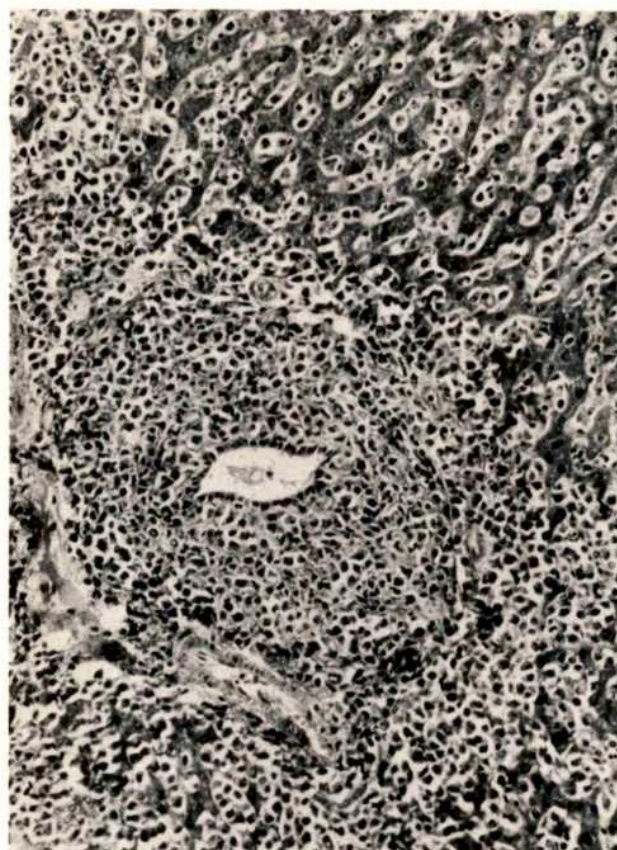


FIG. 5 Tumour infiltration of portal tract and sinusoids in liver of rat heated at 42° C for 1 h, 8 d after implantation of Yoshida tumour into the leg. Death occurred 6 d later. There was also metastatic tumour in the heart, and widespread tumour throughout the retroperitoneal tissues involving the bowel wall and renal parenchyma. Haematoxylin and eosin $\times 120$.

in metastatic distribution of the cancer—the so-called 'soil' originally discussed by Paget²². Histological examination is a relatively crude method of detecting the presence of metastases^{23,24}; it is therefore possible that the present results reflect stimulation of premature activity in Yoshida cells already present in metastatic sites at the time of heating. The appearance of metastases in the liver of two of the sixteen heated animals would seem to argue against this interpretation. A possible depression in the immune defence mechanisms of the host at increased body temperature¹⁰ leading to more rapid spread of the disease must also be borne in mind.

In our work, the Yoshida tumour behaved as a highly-malignant rapidly growing sarcoma, with a capacity for metastasis not commonly found in transplantable animal tumours, but more usually associated with malignant neoplasia in man¹⁹. Although the various factors controlling the selective localisation and development of secondary tumours have not yet been clearly defined, the pathogenesis of a given neoplasm is influenced by a balance operating between the growing tumour and the reaction of the host²⁵. Depending on the adequacy of heat application to the primary tumour, this balance can be altered to the benefit or detriment of the host. It has been claimed that hyperthermia is most effective against rapidly growing tumours of mesenchymal origin^{1,2,8,15}. In the light of our present experiences, however, the possible hazards of inadequate heating of malignant tumours in animals and in man must be considered.

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Recovery of immune system after cigarette smoking

RECENT evidence has shown that the immune response in human cigarette smokers¹ and in mice chronically exposed to fresh cigarette smoke²⁻⁴ is impaired. It has been proposed that depressed immunity in smokers may therefore be involved in the increased prevalence of respiratory infection and neoplasia associated with this habit⁵.

In this laboratory, mice exposed to cigarette smoke show a depression in the development of antibody-producing cells in the spleen, the lungs and their draining lymph nodes, after the introduction of sheep erythrocytes (SRBC) into the respiratory system^{2,3}. Both primary and secondary responses are depressed. This is accompanied by a loss in the phytohaemagglutinin (PHA) responsiveness of the lymphocytes, particularly in the lymph nodes of the respiratory tract⁴. Moreover, the smoke-exposed mice developed an increased number of macrophages in the lungs⁶ similar to that observed in human smokers⁷. Although an infiltration

of lymphocytes into the lungs also occurs in mice soon after the start of exposure to smoke, it is only transient⁸.

The lungs and livers of mice exposed to the smoke also show elevated activity of aryl hydrocarbon hydroxylase⁹, an enzyme which also exhibits high activity in the tissues of human smokers⁹. In other experiments¹⁰, alveolar macrophages lavaged from mice immediately after exposure to smoke exhibited depressed viability, and surviving cells showed altered metabolic activity, indicating penetration of active components of cigarette smoke deep into the lungs. Furthermore, histological sections of lungs from these exposed animals show tar-laden macrophages in the alveoli.

The impairment in the humoral immune response appears to be more complex than just a depression in the amount of immunogen absorbed through the respiratory tract. The antibody-producing cell response at a distant site, the spleen, is less sensitive to the effects of cigarette smoke than sites associated with the respiratory system². A defect in lymphocyte function, as indicated by their impaired responsiveness to PHA (ref. 3), provides a possible mechanism.

While it is important to determine the severity and the mechanisms by which immunosuppression is induced by cigarette smoke exposure, it is equally important to investigate whether or not these effects are reversible on termination of cigarette smoking. We present evidence here which indicates recovery of humoral immunity on termination of cigarette smoke inhalation.

Groups of mice were exposed to fresh cigarette smoke in a Hamburg II small animal smoking machine (Heinr. Borgwaldt, West Germany) on week days. Smoking conditions were as previously described². To measure the humoral immune response, mice were inoculated intratracheally with 10⁸ SRBC, and after 7 d the direct and indirect plaque-forming cells (PFC) in the spleen, the lungs and a pool of the mediastinal and cervical lymph nodes were determined by the Cunningham-Szenburg modification of the Jerne plaque technique as previously described². The

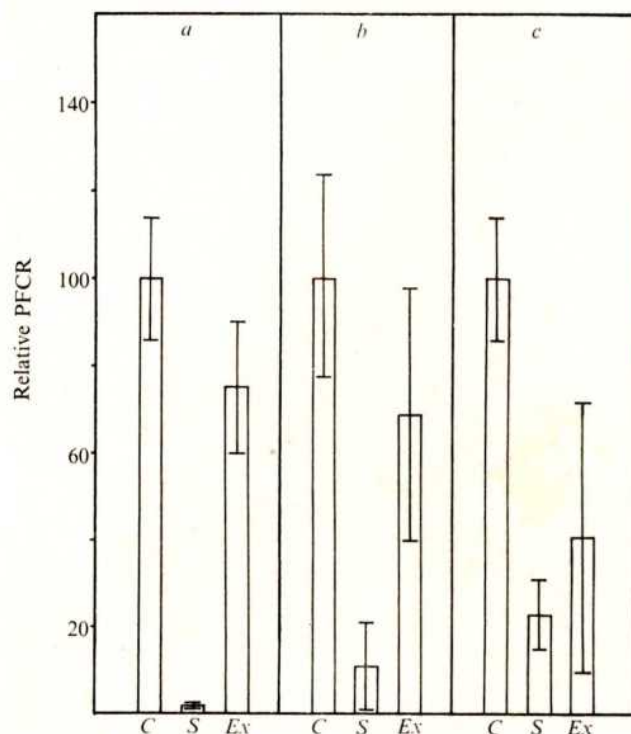


Fig. 1 Recovery of the immune responses of mice to sheep erythrocytes following the cessation of smoking. The plaque-forming cell response (PFCR) before (S), and after, Ex, cessation of cigarette smoke exposure is shown (mean \pm s.e.) as a % of age-matched controls C. a, Lung; b, lymph node; c, spleen.

number of PFC per 10^6 leukocytes was calculated. About 10^8 , 10^7 and 10^6 leukocytes (predominantly lymphocytes) per cell preparation were obtained from the spleen, lymph nodes and lung respectively. No difference between the number of leukocytes in the cell preparations from normal mice and mice exposed to smoke could be detected. The assay time used coincides with the peak primary response of normal mice. Groups of five to nine mice were used in all experiments and the significance of differences between groups was determined by Student's *t* test.

The direct PFC response in mice exposed to fresh cigarette smoke for 42 weeks (*S*), together with the response of mice exposed to fresh cigarette smoke for 42 weeks followed by a further 16 weeks during which time they were not exposed to smoke (*Ex*), are shown as % of age-matched controls (*C*) in Fig. 1. The control mice had an average of 21 PFC per 10^6 leukocytes in the lung, 80 PFC per 10^6 leukocytes in the lymph nodes and 10 PFC per 10^6 leukocytes in the spleen.

Control mice used here were not sham-smoked, as observations^{4,11} indicate that handling stress does not contribute significantly to the effects observed in the exposed mice.

The primary immune response in all organs was markedly depressed after 42 weeks smoke exposure compared with control animals (lungs, $P < 0.05$; lymph nodes, $P < 0.01$; spleen, $P < 0.05$). Sixteen weeks after the cessation of smoking the mice exhibited significantly increased direct PFC responses in the lungs ($P < 0.001$) and the lymph nodes draining the respiratory system ($P < 0.05$) when compared to animals at the end of the 42 week exposure period. Although the average response in the spleen indicated recovery the results observed were extremely variable, with several of the animals still exhibiting low responses. The indirect PFC responses also showed a depression after 42 weeks' smoke exposure in all organs and a recovery 16 weeks after exposure to smoke ceased. The numbers of indirect PFC in the primary responses, however, were low.

The immune system in the respiratory tract exerts a protective function against infections^{12,13} and tumour development¹⁴⁻¹⁶. These diseases show increased prevalence rates in smoking mice^{11,17} and in human smokers¹⁸, but human epidemiological studies indicate that cessation of smoking progressively leads to a lowering of the risk of their development^{18,19}.

If the immune system of man reacts to cigarette smoke in a similar way to that of the mouse, as some data imply¹, the phenomenon of 'recovery' in the ex-smoker^{18,19} may be attributable, in part, to a similar restoration of immune function as that demonstrated here.

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Associative learning of *Drosophila melanogaster*

THROUGH the work of Benzer^{1,2}, attention has been focussed on behavioural studies of *Drosophila melanogaster* and the possibilities of a genetic approach to the investigation of nervous systems. For example, experiments on phototaxis¹ and optomotor response^{3,4} have made possible the selection of behavioural mutants in which electrophysiological^{5,6} and neuro-anatomical⁷ correlates could be established. With the analysis of the mechanism of information storage and(or)

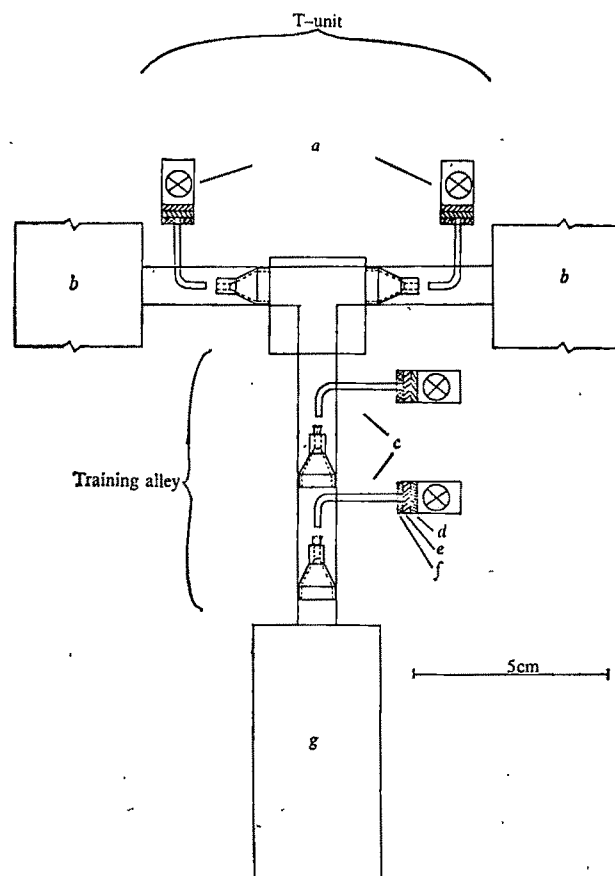


Fig. 1 Schematic drawing of the test apparatus. *a*, lamp; *b*, collecting container; *c*, light conductor; *d*, heat absorption filter; *e*, plexiglass filter; *f*, PVC (grey); *g*, start.

TABLE 1 Results of electroshock experiments in *Drosophila melanogaster*

Experiment No.	Electroshock at funnel No.	Number of 'blue' choices	Number of 'yellow' choices	% 'blue' choices	Δ
I	1 (yellow)	95	103	48.0	+ 9.2
	—	69	109	38.7	
	2 (blue)	50	91	35.5	— 6.9
II	—	61	83	42.4	
	2 (yellow)	131	170	43.5	+13.8
	—	84	199	29.7	
III	1 (blue)	56	98	36.4	— 9.5
	—	45	53	45.9	
	2	94	103	47.7	+ 2.3
IV	—	89	107	45.4	
	1	84	88	48.8	— 0.7
	—	101	103	49.5	

The lamp voltage used in experiments I and II, and for the lamps in the T-unit in experiment III, was 4.0 V.

learning as our ultimate goal, we have attempted to demonstrate associative learning with respect to optical stimuli in *Drosophila*. Here we report our first successful experiments.

Attempts to demonstrate spatial discrimination learning of *Drosophila* were reported by Murphey^{8,9} but later contradicted by Yeatman and Hirsch¹⁰. Recently Quinn, Harris and Benzer¹¹ have found conditioned behaviour in *Drosophila melanogaster* both with olfactory and visual discrimination. Götz (personal communication) has trained individual *Drosophila* with respect to light-dark stimuli. *Drosophila* strains wild type (Berlin) and *ebony* were kept on standard media at 22° C with a 12 h light: 12 h dark cycle. The flies used in the experiments were aged between 1 and 10 d after eclosion (No separation of male and female flies has been undertaken as yet). Because *ebony* have a higher activity in the maze used, experiments were performed with strain *ebony* unless otherwise stated.

The test apparatus (Fig. 1) is related in design to the T mazes used by Hadler¹². It rests horizontally on a table and is constructed entirely of plexiglas. The flies are placed in a starting container and can move freely into the 'training alley'. This consists of a tube 13 mm in diameter with two narrow funnels placed 3.5 and 7 cm respectively from the start. The funnels are constructed (Fig. 2) to provide a one-way passage for the flies. In front of each funnel is a plexiglas light conductor, connected to an Osram 2403 (6 V, 1 W) lamp equipped with plexiglas coloured filters (Röhm and Haas No. 300 and 627). Except for the front faces, the light conductors are covered with black tubing. The flies can thus be exposed to a blue or a yellow light, most intensely at the exit of the funnels. After having passed through the training alley the animals reach a 'T-unit' where they can choose to proceed through a yellow or through a blue illuminated funnel constructed and illuminated exactly as in the training alley. After having passed through these funnels the flies are collected in two separate containers and counted conventionally.

Training is carried out with electroshocks. For this purpose the funnels in the training alley are equipped with tiny copper wires (Fig. 2) connected to the output of a pulse generator. The pulses have a duration of 1 ms with 80 V peak voltage at 80 ms intervals. If a fly touches two wires of opposite polarity, it experiences electrical shocks with currents of about 10 μ A. The animals clearly try to escape these electroshocks. The construction of the shocking device is the critical part of the apparatus. As recorded with a microammeter only about 30% of the animals, especially the smaller ones, can pass the funnel without being shocked.

In the basic experiment, the flies were exposed to an electroshock at only one of the funnels (either blue or yellow illumination) and the % of animals that passed the 'blue funnel' in the T-unit, relative to the total number that completed the run was determined. This was compared to the % measured without application of electroshocks, that is, the spontaneous distribution measured in a separate experiment in identical optical and geometrical conditions but with a new population of flies. To ensure true difference measurements, this control was always carried out immediately before the test run. Furthermore the apparatus was taken apart, washed with detergent and carefully dried between two different experiments, but not between the control and test runs. In this way slight changes in the apparatus after reassembly, which are reflected in the spontaneous tendency (see Table 1), cancel out in the difference between the two runs.

The experiments were carried out at room temperature. Wet filter papers placed in the counting containers helped to maintain an appropriate humidity for the flies and possibly served as attractant as well. Approximately 300 flies were placed in the starting container for each run. At the end of the run, after about 5 h, more than 50% had passed the T-maze. Except for the yellow and blue lamps, the experiments were carried out in the dark. If observation was necessary, overhead red safety lights (Philips TL 40W/15) were used.

For each run the number of animals having chosen the

TABLE 2 Statistical significance of results.

Strain	Electroshock at	Δ	t-Test	χ^2 -Test	Interpretation
Wild type	'yellow'	+10.7 \pm 3.4	$P < 0.01$	$P \ll 0.001$	Avoidance of 'yellow'
	'blue'	-17.6 \pm 5.2	$P < 0.01$	$P \ll 0.001$	Avoidance of 'blue'
<i>ebony</i>	'yellow'	+ 9.1 \pm 2.9	$P \ll 0.01$	$P \ll 0.001$	Avoidance of 'yellow'
	'blue'	-12.1 \pm 2.9	$P < 0.01$	$P \ll 0.001$	Avoidance of 'blue'
<i>ebony</i>	Funnel No. 1 (no lights in training alley)	+ 1.1 \pm 4.4	$P > 0.6$	$P > 0.5$	—

The first four experiments listed used the same arrangement of lights. The first funnel was illuminated with yellow light, the second with blue light. The lamp voltage for experiments with wild type is 3.2 V. *Ebony* is a phototactic mutant; a lamp voltage of 4.0 V was used in experiments with *ebony*. The last experiment listed used no lights in the training alley, but blue and yellow illumination of the funnels in the T-unit.

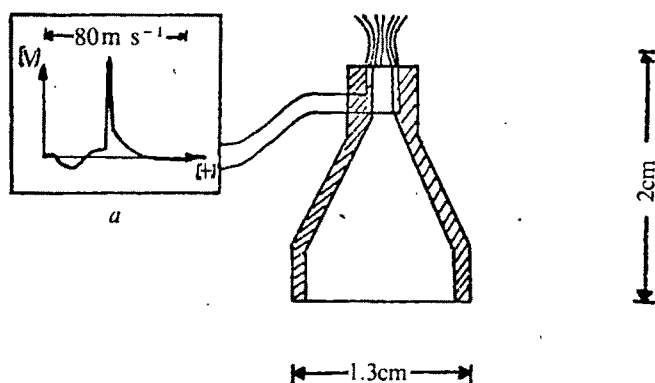


FIG. 2 Schematic drawing of the electroshock device. a, Pulse generator.

blue or yellow illuminated arm of the T-unit were counted and the % of 'blue' choices determined. As we have pointed out above, the experiments were performed as difference measurements between a population having experienced electroshocks associated with a particular optical stimulus and a population which had received no electroshocks. A conditioning index can thus be defined as

$$\Delta = (\text{choices})_{\text{shocked}} - (\% \text{ 'blue' choices})_{\text{control}}.$$

This difference is zero, if the animals do not learn. Avoidance of 'yellow' after electroshocks results in positive numbers, avoidance of 'blue' after electroshocks results in negative numbers.

For a series of these measurements the average of Δ and the standard deviation (not the standard deviation of the mean) was computed (Table 2). A *t*-test for significance of the differences ($\Delta \neq 0$) was performed. Although not quite appropriate, because it neglects the explicit performance of difference measurements, a χ^2 -test of the total number of flies within a series of experiments is listed as well.

Table 1 lists the results from four different sets of experiments. In the first set, the flies were exposed in the training alley to first the yellow light then the blue light. Electroshocks were applied at either the first or the second funnel. In experiment II the order of lights in the training alley, was reversed. In experiment III there were no lights in the training alley, but lights in the T-unit, and experiment IV was run in complete darkness. This last experiment was meant to demonstrate that, with the exception of the light stimuli, the apparatus is completely symmetrical; no right-left bias was observed.

Table 1 lists the number of animals counted in a single experiment and its corresponding control without electroshock. The data are representative of the experiments performed. (Their statistical significance is evaluated through Table 2.) The data suggest that positive learning, that is, association of the optical stimulus with electroshocks and subsequent avoidance of a similar optical environment occurs. The results of these experiments cannot be explained by sensitisation by electroshocks. Because of the symmetrical experimental design, electroshocks both at 'blue' and at 'yellow', a pseudolearning control is unnecessary. That it is indeed the optical stimulus which is being learned is suggested by experiments III and IV which are not significantly different from their respective controls.

Table 2 shows the averages of a number of experiments with wild type *Drosophila* (strain Berlin) and the mutant *ebony*. The data clearly demonstrate a positive learning behaviour both for avoidance of the yellow light stimuli and for avoidance of the blue light stimuli. Although the effects are not large, the deviation from zero of the difference between experiment and control is highly significant in all cases. A sign test yields a high degree of significance. Out of 46 experiments only two cases of 'masochism' were found,

while 44 cases indicated avoidance of the optical stimulus associated with the electroshock. In contrast, if the electroshock was not associated with a light stimulus (no lights in the training alley) no significant differences between experiment and control were found (Table 2).

The greater incidence of positive learning for the wild type in blue light probably results from a favourable relation between light intensity and physiological sensitivity in these circumstances. Further studies are necessary before more quantitative relationships can be established. It is to be expected though, that larger effects will be obtained if the flies are exposed to more than one training situation either at short intervals or on consecutive days.

It should be pointed out that the two light stimuli used in these experiments differ not only in colour but also in physiological rating. Thus, the experiments are not necessarily interpreted as proof of discrimination of the two colours¹¹. There is independent evidence for colour vision of *Drosophila* obtained by Schümperli¹³ as well as in this laboratory. However the data presented here are considered proof of associative learning in *Drosophila* with respect to optical stimuli.

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Identification of chemicals of snail origin that attract *Schistosoma mansoni* miracidia

MEASURES to control schistosomiasis (bilharziasis) aimed at either the molluscan vector or the trematode's larval stages might be enhanced by a better understanding of the intricacies of how miracidia and cercariae find and penetrate hosts. Previous work from our laboratory identified chemicals from mammalian skin that initiated penetration responses by *Schistosoma mansoni* cercariae¹. Other earlier studies implicated amino acids and additional compounds as attractants of *S. mansoni* miracidia to the molluscan host, but did not show that these substances were derived from the snail². Subsequently, based on Neuhaus³ and C. A. Wright's⁴ indications that snails release substances into water that stimulate miracidia, several investigators⁵⁻⁸ have confirmed that snails 'condition' water making it stimulatory or attractive to miracidia. Wright and Ronald⁹ recently demonstrated that water conditioned by the snail *Lymnaea palustris* attracted miracidia of the rodent blood fluke, *Schistosomatum douthitti*, and that this snail-conditioned water (SCW) contained amino acids. Here we show that *Biomphalaria glabrata*, the molluscan vector of *S. mansoni*, the important parasite of humans, contributes amino acids

TABLE 1 Amino acids identified in snail-conditioned water.

Amino acid	($\mu\text{mol l}^{-1}$)
Aspartic acid	0.075
Threonine	0.075
Serine	0.382
Asparagine/glutamine	trace
Proline	0.052
Glutamic acid	0.046
Citrulline	0.028
Glycine	0.124
Alanine	0.153
Valine	0.055
Methionine	0.008
Isoleucine	0.033
Leucine	0.046
Tyrosine	0.025
Phenylalanine	0.027
Ornithine	0.015
Lysine	0.019
Histidine	0.014
Tentative:	1.177
Taurine = $0.054 \mu\text{mol l}^{-1}$;	
Urea = $2.51 \mu\text{mol l}^{-1}$.	

Data represent means of two assays, each on a separate preparation of SCW. Amino acid concentrations were determined from standards prepared in the residue from 500 ml of spring water.

to the aqueous environment, and that these, and possibly other substances elicit chemotactic and chemokinetic responses by miracidia of this parasite.

The schistosome and hosts were maintained as described^{1,2}. SCW was prepared by placing 100 snails (10 mm diameter minimum) in 500 ml of commercial spring water (Magnetic Springs Water Co., Los Angeles, California) for 2–3 h to minimise faecal output during the conditioning process, then rinsing with three 10 min changes of spring water to remove adherent materials. These snails were subsequently used to condition 500 ml of spring water for 18 h. No antibiotic was added to the spring water during conditioning to preclude the induction of amino acid release by the antibiotic. The snails were removed, the SCW filtered (0.45 μm pores), evaporated to 18.5 ml, acidified, and refiltered. The amino acid fraction was eluted from Dowex 50, evaporated to dryness, and taken up in 1.5 ml of 0.2 N sodium citrate buffer (pH 2.2) for analysis. Nineteen common amino acids were identified with an automated analyser (Table 1). No amino acid was detected in controls of 500 ml spring water (no snails) treated as above (three replicates). Two additional control samples were assayed similarly, except that a standard containing a known amount of each amino acid was added to the evaporated spring water. Neither amplification nor increase in numbers of the known peaks was detected. These results clearly demonstrated that the snails contributed amino acids to the water.

The stimulatory or attractive qualities of SCW before and after evaporation was tested by the method of Chernin⁷ and always gave an immediate, strongly positive response. As this bioassay neither yields quantitative data nor necessarily shows a chemotactic response, we devised a choice-system bioassay consisting of a rectangular chamber (37 \times 3 \times 2 cm deep) mounted on the stages of two stereoscopic microscopes. The chamber contained 25 ml spring water. Miracidia (1–4 h old) were added in 1 ml of spring water to the centre of the chamber. For 20 min, two observers simultaneously recorded the number of miracidia entering the 3 cm field of view from the mid-chamber region on each end of the chamber; entries from the distal ends of the chamber were disregarded. This experimental design minimised the number of miracidia in these data that might be 'trapped' by scoring only those that entered from the mid-chamber region. The diffusing chemical may have established an active zone beyond the field of view and in this way caused miracidia leaving the mid-chamber region of

the field of view to return to the source of the stimulus. Such a 'turnback' response indicates chemotaxis^{2,7}.

The time period began when 75 μl of substances to be tested were gently pipetted onto the surface at the centre of each field of view. Experiments were conducted in a darkened room at 22° C. Uniform, diffuse light was supplied by a 10 W neon tube centred 23 cm above the chamber's long axis. Preliminary experiments designed to standardise the system showed that this method gave a dynamic, quantitative analysis through direct observation of miracidial behaviour in the presence of both experimental and control substances.

Using this system, we demonstrated that the presence of SCW produced significantly more entries into the field of view than did spring water controls (Table 2). In addition, behavioural changes described by MacInnis² and by Chernin⁷ were exhibited by nearly all miracidia entering the field containing SCW, but rarely in the control. In other experiments concentrated SCW was prepared in agar blocks², then bioassayed in the chamber against agar made with spring water. At the end of five 10-min experiments with these agar blocks, the ratio of miracidia in the fields of view was 10:1 (experimental:control). These results clearly demonstrated that the presence of SCW not only attracted miracidia, but also kept more miracidia in the vicinity of the source of the stimulus.

To ascertain whether or not the amino acids identified in SCW could elicit responses by miracidia, a mixture of amino acids was prepared at concentrations equivalent to that determined to be present in SCW (Table 1, taurine and urea were omitted). This mixture was also assayed with the amino acid analyser, and the concentration of each amino acid shown to be essentially as noted in Table 1. Bioassay of this mixture (Table 2) demonstrated that the amounts of amino acids produced by snails increased the number of entries into the field of view. This mixture appeared slightly, but not significantly, more effective than the SCW assayed (Table 2, $P > 0.1$). This difference might be explained by the fact that the size of snails used to prepare SCW in these trials was somewhat smaller than those used to prepare the SCW for chemical analysis. Such an interpretation is consistent with Chernin's results with SCW from snails of different sizes⁷.

Table 2 also shows the results of double control experiments in which 75 μl of spring water were added to each end of the chamber. Comparison of these results with those of the controls run with SCW or the amino acid mixture demonstrated that the presence of the SCW or amino acids not only increased entries into the field of view, but also reduced the number of entries in the field opposite the chemicals. Such results indicate that amino acids and other chemicals released by snails cause miracidia to localise preferentially in

TABLE 2 Bioassay of 75 μl of snail-conditioned water and of an amino acid mixture equivalent to the amino acids determined to be present in it (Table 1).

Time (min)	Entries into field of view (mean \pm s.e.)			
	Control	SCW	No.	P
10	22 \pm 2	38 \pm 6	(5)	<0.05
20	55 \pm 4	78 \pm 5	(4)	<0.05
	Control	Amino acid mixture		
10	21 \pm 2	34 \pm 4	(6)	<0.02
20	54 \pm 2	89 \pm 4	(6)	<0.001
	Control	Control		
10	23 \pm 3	23 \pm 3	(5)	>0.80
20	61 \pm 6	65 \pm 7	(5)	>0.60

No., Number of experiments (50 miracidia per experiment in 25 ml spring water); control is 75 μl of spring water; in the double control experiments 75 μl of spring water were added to each end of the chamber. Replicates were accomplished by alternating experimental and control additives in each end of the chamber. Probability, P , calculated from Student's t test.

the region of the stimulant, and thus enhance the chances of a miracidium encountering a snail.

SCW contains many components other than amino acids. Preliminary experiments with a peptide fraction (obtained from SCW by the method of Nakano and Yamamoto¹⁰) showed no attraction over a distance, but did elicit slight modifications of the normal miracidial swimming pattern. Preliminary analyses of SCW (transesterified with methyl alcohol) by gas-liquid chromatography indicated the presence of at least six peaks whose identity and ability to stimulate miracidia have not yet been resolved. Our results with the amino acid mixture, and those of Shiff and Kriel⁶, who passed their SCW through a 'mixed-bed resin', suggest, but do not preclude the possibility, that inorganic ions need not contribute to attraction of miracidia.

Although amino acids or other substances may leach out of the mucus and faeces of the snails, amino acids also may be released from the epithelial cells of snails in an osmoregulatory function as discussed by Potts¹¹. It may be noted that Chernin⁷ reported that faeces of *B. glabrata* were not attractive to *S. mansoni* miracidia in the bioassay. C. A. Wright⁴ found that faeces did stimulate miracidia, but such results must be interpreted with caution as any material removed from SCW may absorb sufficient amounts of the stimulative substances to elicit a response. Our results clearly proved, however, that the amino acids produced by snail hosts can serve as attractants for miracidia. How can such results be used to improve methods of controlling bilharziasis and other pathogens?

It is possible that amino acids (and other, as yet unidentified components of SCW) produced by snails, or released from plant materials during feeding by snails, keep miracidia, cercariae and snails within a limited focus helpful to completion of their life cycles. Attraction of snails to amino acids was proposed by MacInnis, but has not yet been tested².

Incorporation of attractants such as amino acids into larvacides and molluscicides, possibly into easily dispersed materials such as proposed by N. F. Cardarelli (United States Patent No. 3,417,181, December 17, 1968) may be used to attract both parasite and vector into a zone of effective pharmacopoeia. Alternatively, they might be attracted to particles containing insoluble poisons (which would be less dangerous to the ecological aspects of the environment) and induced in the case of the mollusc to feed upon such materials, or, in the case of the larval trematodes, to waste their energies and secretions in futile attempts to penetrate particles emanating attractants which also initiate penetration responses^{2,7}. Amino acids and other substances released from processed plant material used as a schistosomacide or molluscicide, might also contribute to the success of such material when used in nature^{12,13}. These possibilities, in view of the fact that the widely used molluscicide sodium pentachlorophenate is a repellent to snails¹⁴, further provide support and impetus for future investigations on artificial or natural larvacides and molluscicides in combination with attractants.

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Stereopsis by binocular delay

HERE I report results which reveal a new form of binocular stereopsis sensitive to delays between the arrival of common input to both eyes instead of retinal disparity.

Until the work of Julesz¹ monocular form detection was commonly believed to be a prerequisite for the detection of binocular retinal disparity and so for stereopsis. Julesz showed monocular form detection not to be necessary by demonstrating cyclopean perception of targets in depth with random dot stereograms. Neither member of the stereogram pair conveyed any monocular information about the target. A continuous analogue of Julesz's random stereogram method is used in the present observations to show that neither monocular form nor binocular disparity is necessary for stereopsis.

Figure 1 shows that when both eyes are fixated on a target, each sees different areas of the foreground and background, a fact established by Hering's² demonstration of the Law of Identical Visual Directions. It follows that as the eyes rove at a constant fixation distance, they encounter the same background texture at different times, the delay being a function of the eyes' rate of traverse, the distance of the target and the distance of the background.

When they move from left to right, the left eye encounters parts of the background before the right. In the foreground, the situation is reversed. The right eye leads and the left trails. Both eyes encounter whatever is at the fixation distance at exactly the same time.

The apparatus presented each eye with some common (target) input at the same time, and some common (background) input at different times. Each eye viewed a point-plotting oscilloscope (Tektronix 602, P15 phosphor) controlled by a PDP8-E computer via specially designed interfaces. (For details see Ross and Hogben³.) Polarised filters shield each eye from the other's view. The first target was an outline square (2 cm × 2 cm), presented to each eye simultaneously in repeated 10 ms bursts, at a rate above critical flicker fusion. The background was a continuous (or dynamic⁴) noise stream of single dots, distributed uniformly at random over the whole of an 8 cm × 8 cm display area, within which the target was centred. Dots were plotted at a rate of about 9 ms⁻¹ whenever the target was not being plotted, so that each eye seemed to see a field of many dots, like a snowstorm, the distribution continuously changing. Each dot in the stream was shown to both eyes at the same rate, but at different times. One stream, so to speak, lagged behind the other.

It should be noted that delay here does not produce an effective disparity, as it would if successive points fell in order along a horizontal line, because points fall randomly in two dimensions. Effective disparity due to delay has been proposed as an explanation for the Pulfrich phenomenon.

When the delay between the eyes is below about 50 ms, the binocular view reveals a target roughly in the same plane as the noise field. When it is above about 70 ms, the noise field recedes well behind the target. The effect is vivid, and unmistakable, even for some observers with poor stereopsis for displays based on disparity. Between 50 and

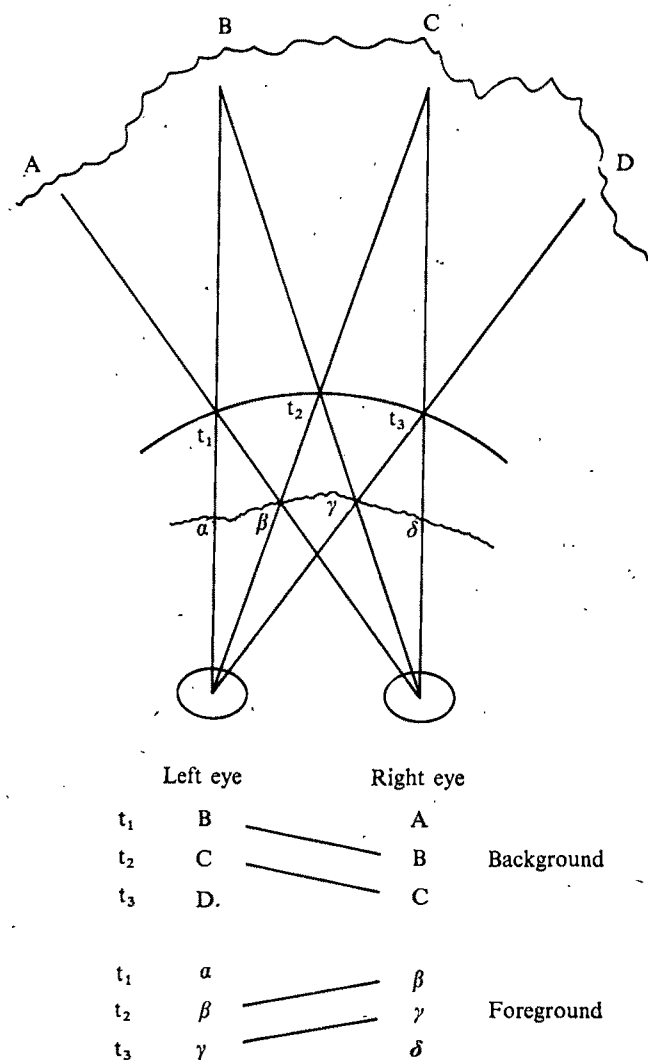


FIG. 1 View of each eye at different times. A, B, C and D are points in the background; α , β , γ and δ are points in the foreground. Because of motion parallax, rate of transverse over the background is more rapid than over the foreground.

70 ms, the situation is ambiguous, the background being seen alternately in the plane of the target, or behind.

Delay was pitted against disparity by shifting dots in the noise stream to give them a crossed disparity. Below 50 ms delay, disparity is dominant, the noise plane being seen in front of the target, and transparent. Above 70 ms, delay is dominant, the noise plane now lying behind the target and looking opaque. In the 50–70 ms range, the background alternates from in front to behind. The alternation does not seem amenable to voluntary control.

The monocular contour provided by the outline square can be eliminated by an analogue of the Julesz method, mentioned above. Points to be plotted to both eyes are generated at random within the 8 cm \times 8 cm available display area. Points falling within a central square area (2 cm \times 2 cm) are plotted, forthwith and simultaneously to both eyes. Points falling outside the central square area are plotted immediately to one eye, and buffered, to be displayed, at the same rate, but later, to the other eye. Each eye sees the same points at the same rate and distributed uniformly at random over the whole display area. All that differs is the time of presentation. There is no monocular distinction between target and background. The only mechanism which could determine that there was a distinction is one which had access to both fields of view, and which could register and recognise differences between them.

When delay in the background exceeds 70 ms, the target is seen standing in depth, in front of the background. The edges of the target are not so sharply delineated as when disparity is used to define a target but, as with disparity, there is no apparent motion of dots across the boundaries of the target. (See Julesz¹, and Ross and Hogben³, for comparable effects with disparity). Of course, there is no similar confinement of motion within either monocular view. At delays below 50 ms, the target can be distinguished from the background, but not clearly and only faintly at differential depth.

When the background is composed of independent sets of points, so as to produce rivalry, both targets, the monocularly visible and the monocularly invisible, sit embedded in, rather than in front of a nebulous surround, thus eliminating the possibility that delay induces complete or partial rivalry and has its effect accordingly.

The observations reported here show that retinal disparity determines relative depth when input is received by the two eyes within a span of 50 ms, confirming the value determined by Ross and Hogben³, for short-term memory in stereopsis. More importantly they show that delay alone can determine relative depth when the span for disparity is exceeded. It can do so even in the absence of monocular information about form, as can disparity.

The upper limit for delay has not yet been determined precisely, but indications are that it is near 2 s. At delays beyond 2 s the target is visible, but the background looks rivalrous, has the nebosity characteristic of independent random point streams and is no longer a plane behind the target.

Many observational details fit the suggestion that the visual system handles common input with binocular delay as if it had been generated by eye movements (see Fig. 1). When the left eye receives input first, the background streams from right to left, as if the eyes were sweeping left to right. The target appears stationary. Direction of background movement is reversed when the right eye receives input before the left. At certain delays, there is also a lacy plane in front of the target, with movement always in a direction opposite to that on the back plane. As Fig. 1 shows, the eye leading in seeing background texture trails in seeing foreground texture.

Retinal disparity provides a fine sense of depth within a narrow spatial range, Panum's fusional area, and a narrow temporal span of about 50 ms (see Ross and Hogben³). We may speak of a fusional area and a fusional span, both limiting disparity in stereopsis. The results reported show that the visual system can also deduce relative depth, and direction of movement, from common input received at binocular delays beyond 50 ms. There thus seem to exist two binocular systems for depth, one operating within narrow spatial and temporal limits, to give fine resolution around fixation, and the other operating outside the temporal limits of the first to give a coarse indication of the structure of background and foreground space.

This research is supported by a grant from the Australian Research Grants Committee. I thank A. Sala, who designed much of the apparatus, and J. H. Hogben, who helped in programming and in clarifying the displays.

JOHN ROSS

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Received October 29, 1973.

¹ Julesz, B., *Bell System Tech. J.*, **39**, 1125 (1960).

² Hering, E., *Outlines of a Theory of the Light Sense*, Trans. from German original, of separate sections (Harvard University Press, 1964).

³ Ross, J., and Hogben, J. H., *Vision Res.* (in the press).

⁴ Uttal, W., *Percept. and Psychophys.*, **6**, 121 (1969).

⁵ Julesz, B., *Foundations of Cyclopean Perception* (University of Chicago Press, Chicago, 1971).

science on television

Visceral learning

John Wilson

DR NEAL MILLER, of Rockefeller University, New York, believes that we can learn to control our blood pressure, and other regular bodily functions, just as we control the movement of our limbs—if only we try hard enough. With my overdraft in mind, I thought that my bank manager might benefit from the technique so when Yorkshire Television asked "What's Visceral Learning Anyway?" I settled back to learn the answer.

It was not long in coming. Before the opening credits had rolled there was Dr Miller's most successful hypertensive patient, a woman, telling all. She did not know how she did it, she said, it just happened (or rather more accurately, it later transpired that she provided explanations, such as thoughts of old Bogart movies, which her doctor discounted).

Having given the game away at the outset, the programme went on to relate the history of visceral learning, and did so very well. The phenomenon was introduced by way of examples (a young boy controlling the temperature of his hand, a gunshot victim regulating his blood pressure and Dr Miller himself wiggling one ear only), followed by a brief description of the relative impor-

tance of the somatic and autonomic nervous systems.

Dr Miller, we learned, first came to doubt classical theory on the independence of the autonomic system as a result of some very courageous experiments involving 16 human volunteers. When paralysed from the waist down by injections of curare or acetylcholine, these volunteers could still control their urine. If the bladder could be regulated in this way, he reasoned, might not other usually automatic functions of the body be controlled also?

For his experiments on rats, Dr Miller implanted electrodes into their brains so that he could reward them for a 'correct' response in blood pressure even when they were totally paralysed with curare. In living colour, the surgery involved must have turned off nearly as many sets as the recent party political broadcasts but to those of us inured to this kind of bloodshed it provided a fascinating insight into the procedure involved in this elegant experiment which, incidentally, took three years to develop. It was also good to see the ambiguities of the experimental work being admitted to the public and the importance of control experiments emphasised. In view of the *dénouement* to follow, this was just as well.

For the sombre truth about Dr Miller's experiments is that nobody has ever been able to verify them, although five years

and 2,000 unfortunate rats later another researcher, Dr Barry Dworkin, is still trying. Various reasons for his failure were presented to the viewer and then demolished, just as they were gaining credibility.

Had the company supplying the rats been rearing its charges in too much luxury and thus making them less susceptible to stress? No, when the animals were subjected to what Dr Dworkin called the "summer camp" treatment by dropping them in ice-cold water (heaven preserve me from American summer camps) they continued to turn in contradictory results. Could the curare used in the original experiments have been altered? Apparently not—but perhaps a placebo effect was taking place.

This sort of criticism in a television science programme is rare and could have been handled in a most destructive manner but the "Discovery" team should be congratulated on its balanced view of a most interesting topic.

The programme did contain a rather surprising flaw, however. Against a picture of a row of oriental mystics, seated deep in meditation and with electrodes taped to their shining heads, we were told of their amazing ability to confound modern science, represented by the scribbles of an electroencephalogram. No sooner seen than gone, the achievements of these people surely deserved more than this cursory mention.

matters arising

African swells, magmatism and plate tectonics

SIR,—Gill¹ states that the 'basin-and-swell' topography of Africa is now widely regarded as the product of heat flow anomalies in the upper mantle. Are these anomalies fixed with respect to the Earth's rotational axis or not? My contention is that they are not. The great Mesozoic to late Tertiary magmatic provinces of eastern Africa become progressively younger northward, when the overall movement of the African plate was in general northward and north eastward; and the mag-

matic provinces of west Africa have remained in more or less the same place since mid Jurassic times.

My ice-floe analogy⁴ was intended only to suggest that gentle lithospheric buckling might initiate the development of these broad thermal anomalies, or megaplumes, perhaps by enabling an asthenospheric up-current to develop, which would then be the source of magmatism and the main agent of crustal doming, and which could easily give rise to tensional features, such as rift valleys.

Gill quotes a figure of 10 km as the maximum possible wavelength for 'compressional warp structures', which seems improbable in a lithosphere 70–100 km

thick. I also question whether the idea that gentle warping in response to stresses arising from interplate 'jostling' is invalidated by reference to post-glacial isostatic recovery rates.

It is therefore still not impossible that a small degree of pressure-relief partial melting could trigger development of asthenospheric upcurrents. Gill is correct in inferring that I emphasise the mantle as a source of much salic magmatism, but whether such magmas are produced directly by partial melting or indirectly by high pressure fractionation is not important—what is important is that the mantle is demonstrably the source of some phonolites and trachytes. I still prefer the partial

melting model, and Gill states that such a model requires low temperature salic melts to be sweated off from the mantle earlier than basaltic liquids. In east Africa, great effusions of phonolitic magma were among the earliest manifestations of volcanism. In this connection, it is also worth noting that the difficulties of explaining the simultaneous availability of basic and salic magmas are just as great on a fractionation as on a partial melting model.

Yoder² has outlined a fractional melting mechanism to account for the contemporaneous occurrence of magmas of highly contrasted composition—in particular basalt and rhyolite, although there is no reason why similar arguments could not apply to alkaline basic and salic magmas. In a modified version of his earlier ideas, Bailey³ has proposed that plate tectonic processes could give rise to uneven stress distribution, resulting in gentle warping of continental plates. If low melting constituents were drawn into the arches, the resulting lower density mantle would provide a density contrast for continued uplift as observed in major continental alkaline provinces.

Finally, whether or not these comments constitute a valid reply to the points raised by Dr Gill, the central thesis of my paper is unaffected: alignments of individual magmatic centres within a magmatic province can only very rarely be attributed to movement of the lithosphere over a stationary mantle hot spot.

Yours faithfully,
J. B. WRIGHT

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Bletchley,
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¹ Gill, R. C. O., *Nature*, **247**, 25 (1974).

² Yoder, H. S., *Am. Mineral.*, **58**, 153 (1973).

³ Bailey, D. K., *J. Earth Sci.*, **8**, 255 (1972).

⁴ Wright, J. B., *Nature*, **224**, 565 (1973).

Second Law of Thermodynamics

SIR,—In a recent article¹ Hillel proposes a statement of the Second Law of Thermodynamics which he claims is consistent with the time reversal symmetry of the microscopic laws of physics. Because the issues raised in this article nicely illustrate some of the fundamental misconceptions which continue to bedevil the subject of temporal asymmetry, I wish to make the following response.

In stating his form of the Second Law: "The entropy of a closed system tends to change monotonically with time", Hillel confuses the assumed underlying isotropy of time itself (ab-

sence of intrinsic preferred orientation) with the symmetry of physical processes with respect to time, a confusion which is in fact almost universal. The isotropy of time itself always enables us to replace the words "increasing entropy" by "decreasing entropy" in thermodynamic statements, for this merely amounts to an inversion of our conventions "later than; earlier than"—permitted provided these conventions are not referred to other physical processes (such as K^0 meson decay) which are asymmetric in time independently of thermodynamics. Hillel's remarks are thus a statement about time and not about thermodynamics.

Indeed, his is not a statement of the Second Law consistent with the symmetry of microscopic processes with respect to time, as alleged. For it is precisely the stated monotonic change of entropy in time which is the asymmetry of thermodynamics and which is apparently in conflict with the reversibility of atomic motion (Loschmidt's paradox). The fact that we are free to call the change an increase or decrease cannot alter the fact that the change is still asymmetric in time. Such a situation is well known to be incorrect, because it contradicts Poincaré's theorem, according to which all states in a closed system will be closely revisited eventually. Most laboratory systems soon reach equilibrium, after which fluctuations occur, during which the entropy both increases and decreases. These fluctuations often lead to observable effects such as Brownian motion.

A truly time symmetric statement of the Second Law, which is certainly necessary, has always been available:

If a closed system is in a random state of low entropy it is overwhelmingly likely to have been in a higher entropy state just before that moment and to be in a state of higher entropy just after that moment.

This statement resolves Loschmidt's paradox and is consistent with Poincaré's theorem. It also becomes consistent with our observations when it is appreciated that real systems are not permanently closed, but are branch systems from the main environment formed at a finite time in the past. Such systems are formed in low entropy states, and simply do not exist prior to their formation. Consequently, the monotonic entropy change referred to by Hillel is actually a consequence of the asymmetric formation of branch systems, which itself is related to the condition under which the universe emerged from the "big bang". It is actually possible to understand this condition in detail in terms of reaction thresholds of elementary particle processes on the one hand, and the

peculiarities of gravitational thermodynamics on the other. I refer the reader to my forthcoming book for a full discussion of these cosmological topics².

Yours faithfully,
P. C. W. DAVIES

King's College,
University of London

¹ Hillel, A. J., *Nature*, **242**, 456 (1973).

² Davies, P. C. W., *The Physics of Time Asymmetry* (S.U.P., in the press).

Dr Hillel replies: On the basis of classical dynamics, one can show that a system in a random state of low entropy is overwhelmingly likely to be in a higher entropy state just before that moment and also just after. This is put forward by Davies as a statement of the Second Law which has always been available in the literature. It is, however, a theoretical statement which is still commonly thought to be at odds with the observations which we summarise in the Second Law. This is precisely the basis of Loschmidt's paradox. The theory allows the possibility of a system "prepared" at a certain time, whose entropy was decreasing monotonically at earlier times. This seems to be inconsistent with observations and is forbidden by the usual statement of the Second Law. At this stage it is argued that in practice the system cannot exist before its preparation. I do not find this argument helpful. It does not remove the asymmetry but merely restates it. Is it possible, in principle, to observe such a monotonic decrease in entropy, and if so would the observations be physically acceptable to us? I argue that the answer is yes and that our statement of the second law should be modified accordingly. The statement I propose is consistent with the reversibility of the laws of mechanics (as embodied by Davies's theoretical statement). Poincaré's theorem is not relevant when considering statistical tendencies during times much shorter than the Poincaré time.

I do not accept Davies's distinction between the isotropy of time itself (absence of intrinsic preferred orientation) and the symmetry of physical processes with respect to time. A physical law is symmetrical under time reversal if, for any allowed sequence of events, the time reversed sequence is also allowed by the law. My proposed statement was designed to satisfy this criterion, because thermodynamic observations are symmetrical in this sense, and that is why a thermodynamic time reversal produces no observable effect other than an apparent inversion of our verbal conventions "later than; earlier than".

Schuster Laboratory,
University of Manchester

obituary

G. Scatchard

GEORGE SCATCHARD was born in Oneonta, New York, on March 19, 1892, and died in Cambridge, Massachusetts, where he spent most of his life, on December 13, 1973. He received his AB degree from Amherst in 1913 and his PhD from Columbia in 1917. His PhD degree was in organic chemistry, on the synthesis and properties of quinazolines, but his interest shifted early to physical chemistry, and it is as a physical chemist, or, more precisely, as a thermodynamicist, that he established his reputation. He was Professor of Physical Chemistry at MIT for most of his career.

George Scatchard loved thermodynamics, its pure simplicity of expression, its certainty, its complete freedom from questionable assumptions. The vaguely understood effects of intermolecular interactions are always rigorously included in his equations as activity coefficients and their derivatives. Much of his theoretical work was concerned with the extraction of these non-ideal parameters from experimental data, with their interpretation, and with extrapolation methods that would eliminate them and permit exact determination of the simpler parameters, such as standard electrode potential and molecular weight, that enter into the definition of ideal thermodynamic behaviour. In the laboratory, George Scatchard dedicated himself to valiant efforts to match experimental precision to the precision of the equations he used for analysis. A characteristic of all his work, painfully discovered by many less patient experimenters who have sought to use his methods for analysis of their own data, is his ingenuity in devising plotting procedures that amplify experimental error and reveal at a glance the uncertainty of an extrapolation or in the drawing of a tangent.

George Scatchard was a severe critic. He did not necessarily expect others to match his own precision of thought or experiment, but he expected them to try their best. When he detected gross confusion or misconceptions, and especially when they masqueraded behind a facade of glib showmanship, he could be merciless. He will not be forgotten by anyone who presented seminars at the Harvard-MIT Physical Chemistry Colloquium when he was present. There was a perpetual frown on his face, deepening in intensity at each point where the speaker was glossing over

theoretical or experimental difficulties. At the end of the seminar there was relief when he asked an innocuous question, and (depending on the personality of the speaker) either anger or a silent resolve never to err again when he rose to expose a fallacy. There are undoubtedly some who remember George Scatchard with less than affection as the result of such an encounter, but there are many more who are grateful for his example and for the effect it had of raising their own standards of what is and what is not a valid piece of scientific research. As might be expected, George Scatchard's scientific papers often had the purpose of defining parameters and establishing methodology.

From 1930-40 he was mostly concerned with the thermodynamics of solutions, and especially of aqueous solutions of electrolytes beyond the range of applicability of limiting laws. During this period he developed the isopiestic method for evaluating osmotic and activity coefficient, and wrote definitive papers on the use of electromotive force and freezing point depression. He also wrote purely theoretical papers including a pioneering paper with John G. Kirkwood, during tenure of a Guggenheim Fellowship in Leipzig, on the electrostatic interactions of zwitterions.

During the Second World War, he divided his time between the Manhattan project of the AEC and the blood plasma fractionation project at Harvard directed by E. J. Cohn, who had been a fellow undergraduate at Amherst and was a life-long friend. The latter work led to a general interest in proteins and to his classic papers in 1946 on the state of equilibrium across a semipermeable membrane. These papers were written to provide a sound foundation for osmotic pressure measurements of protein solutions at finite concentrations, but of course apply quite generally to all thermodynamic properties of multi-component macromolecular solutions. Explicit extension to sedimentation equilibrium was made by Scatchard himself in 1954. The same basic theory provided the foundation for Scatchard's experimental work on the binding of ionic ligands to proteins and their interpretation in terms of specific binding sites and association constants, as well as for several papers on transport across semipermeable membranes.

There are very few scientists whose work one can legitimately compare with that of the handful of all-time great in-

tellects. Such a comparison can be made, without appearing presumptuous, between George Scatchard and J. Willard Gibbs. Nor is it derogatory to George Scatchard, in making this comparison, so say that Gibbs led the way and Scatchard followed for Scatchard himself said so. Few have the mental discipline that is required to go beyond a comprehension of what Gibbs accomplished, to an understanding of how he did it, to a sufficient insight into his thought process to be able to emulate it and to apply it to new and complex situations and George Scatchard was unique in his generation in his ability to do so. Without him many of the fruits of Gibbs' work would not have been gathered so soon.

C A Coulson

Just over a century ago, T. H. Huxley wrote a paper in which he described the liberally educated man as one "who has been so trained in his youth that his body is the ready servant of his will and does with ease and pleasure all the work that, as a mechanism, it is capable of: where intellect is a clear cold logic engine with all its parts of equal strength and in smooth working order . . . whose mind is stored with the great and fundamental truths of Nature . . . and who, no stunted ascetic is full of life and fire, but whose passions are trained to come to heel by a vigorous will, the servant of a tender conscience; who has learned to love all beauty, whether of Nature or of art, to hate all vileness and to respect others as himself". It is ironic that an atheist should have so adequately encompassed the life and style of Charles Coulson that, provided 'Christian' is substituted for 'tender' no better words could be found for his epitaph. This remarkable man was born in 1910 and died in Oxford on January 6, 1974, steadfast to the end in his adherence to his principles and beliefs and in his sense of duty to others so that his last concerns were not for himself but for those at home and in his department who were, in diverse ways, dependent on him.

He entered Trinity College, Cambridge, from his local Grammar School as an open scholar and duly and predictably collected his first in the Tripos examinations. His first paper in 1931 was essentially an attempted criticism of one of the redoubtable Harold Jeffreys' conclusions and its writing epi-

tomised his unfettered pursuit of truth even if it led him into conflict with higher authority. In due course he was elected a Research Fellow of Trinity sharing this distinction with two physicists also with Methodist connections, the late L. H. Gray FRS and D. E. Lea. Their scientific interests converged in the then embryonic study of the action of ionising radiation on living systems. Lea and Gray went on to make most distinguished contributions to this field, but Coulson's was not particularly outstanding and he turned to the newly emerged subject of theoretical chemistry, then broadly defined as the application of Wave Mechanics to molecular structure and bonding, which occupied him for the last 37 years of his life successively as lecturer in University College Dundee, ICI Fellow in the Physical Chemistry Laboratory at Oxford, Professor of Theoretical Physics at King's College London, Rouse Ball Professor of Mathematics at Oxford (for about 20 years) and finally as the foundation Professor of Theoretical Chemistry at Oxford.

A combination of enthusiasm, great industry, fluency and an uncanny judge-

ment of when to approximate made him a most prolific author (three books and over 300 papers). He touched on many chemical problems such as aromaticity, conjugation and hyperconjugation, charge distribution, shapes of molecules in ground and excited states. If his role initially was often to provide *post hoc* quantum-chemical justifications for many qualitative and semi-quantitative relations empirically perceived by chemists, he correctly foresaw that, once the computational obstacles had been overcome, the latent predictive powers of quantum chemistry would be realised and that it might outstrip instruments in quantitative precision.

Coulson will perhaps be remembered more as an unusually gifted teacher than a great researcher. He liked young people and they liked him—in their patois he 'related'. He had great sympathy for the intellectual difficulties of beginners and spared no pains in helping them. He loved lecturing and was a brilliant expositor, leaving his audience eager for more and confident that he could help them to an understanding which would enable them to select and solve prob-

lems. His summer schools in theoretical chemistry were world famous and a powerful influence in the dissemination of ideas and the development of the subject. They augmented the already strong attraction of Coulson's department for overseas visitors who often seemed to predominate in his research group.

Obituaries in *Nature* emphasise scientific achievement but it would be unjust to Coulson's memory not to recall that he was a deeply concerned Christian. This was manifested not only in his lay preaching to small congregations in insignificant Methodist Chapels or his public lectures to sophisticated audiences as in his Gifford lectures, but also in his willingness to play very active parts in organisations—such as a Vice-President of the Methodist Conference or as a working Chairman of Oxfam.

Honours were poured on Coulson but are not enumerated here for he would have preferred to be remembered simply as a practical idealist anxious to add to the sum of human happiness and understanding by advancing knowledge and by teaching and helping others, all of which he achieved abundantly.

Announcements

Appointments

J. Bardeen, H. B. G. Casimir, L. E. F. Neel, Sir Rudolf Peierls and Sir Alan Wilson have been elected Honorary Fellows of the Institute of Physics.

Sir Michael Perrin has been appointed a Trustee of the British Museum (Natural History).

Awards

The Council of the Institute of Physics has awarded the Guthrie Medal and Prize to R. L. Mossbauer of the Institut Max von Laue-Paul Langevin; the Rutherford Medal and Prize to A. E. Litherland of the University of Toronto; the Glazebrook Medal and Prize to B. J. Mason of the Meteorological Office; the Charles Vernon Boys Prize to P. G. H. Sanders of the University of Oxford; the Maxwell Medal and Prize to S. F. Edwards of the University of Cambridge and the Max Born Medal and Prize to W. Greiner of the University of Frankfurt.

The Councils of the French Physical Society and the Institute of Physics have awarded the 1974 Holweck Medals and Prizes to A. Hewish of the University of Cambridge and P. Nozieres of the Institut Max von Laue-Paul Langevin.

The Royal Society has awarded the first Herschel Medal to P. Wild of the CSIRO Division of Radiophysics.

Erratum

IN Fig. 1 of the article "Nitrosocarbaryl as a potent mutagen of environmental significance" by R. Elespuru W. Lijinsky and Jane K. Setlow (*Nature*, 247, 386; 1974), the vertical axis should have been labelled mutants/survivor. On line 17 of the figure legend 0.01M NC should read 0.01mM NC.

Corrigendum

In the article, "Mechanism of denaturation of haemoglobin by alkali" by M. F. Perutz (*Nature*, 247, 351; 1974) on page 343, paragraph 3, lines 9 and 10 of the section 'Probable mechanism' should read "Alkali denaturation of human oxyhaemoglobin has a negative entropy of activation . . ." and the first author of ref. 29 was misspelt and should be Air, G. M.

International meetings

April 8-10, 18th Annual Meeting of the Ecological Genetics Group (G. E. Marks, The John Innes Institute, Norwich, UK)

April 8-10, Course on Quantitative Microscopy for Materials Scientists (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA)

April 8-10, 8th Thin Films Conference (The Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 80X)

April 8-11, The Engineering Uses of Coherent Optics (Mr E. R. Robertson, Department of Mechanics of Materials, University of Strathclyde, Glasgow)

April 8-12, International Symposium on Wound Healing (Secretariat International Symposium on Wound Healing, c/o Holland Organising Centre, 16, Lange Voorhout, The Hague, The Netherlands)

April 9, Society for Drug Research Symposium on Essential Hypertension (Dr A. B. Simmonds, Chelsea College, Manresa Road, London SW3)

April 9-10, Microstructure and Physical Properties of Non-metallic Materials (Dr D. Dew-Hughes, Department of Physics, University of Lancaster, Lancaster)

April 9-11, Gels and the Gelling Process (The Faraday Division, The Chemical Society, Burlington House, London W1V 0BN)

April 10, Applications of Remote Sensing—Data Processing and Analysis (R. W. Laing, Electro-Optics Division, Hawker Siddley Dynamics Ltd., Manor Road, Hatfield, Hertfordshire AL10 9LL)

April 15-16, 11th Annual Rocky Mountain Bioengineering Symposium (Clifford D. Ferris, The University of Wyoming, College of Engineering, Laramie, Wyoming 82070)

nature

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The French: are they legal?

It is ironic that at a time when Professor Jacques Monod has to go to extraordinary lengths to keep the Institut Pasteur alive, the French government should be spending several hundred million pounds annually on nuclear defence. The continuance of a vigorous and controversial French programme of atmospheric weapons testing is bound to stimulate more argument this summer both within France and beyond her borders and so the appearance of a summary by the Stockholm International Peace Research Institute (SIPRI) of the legal questions of French activities is a welcome event (*French Nuclear Tests in the Atmosphere: The Question of Legality*, SIPRI, Stockholm, March 1974).

France started her military nuclear programme in the 1950s and by 1960 was able to test fission devices in Algeria, at first in the atmosphere but from 1961 underground. In 1966 testing was moved to the South Pacific and has continued at the rate of several devices a year in the atmosphere since then.

Concerted attempts to stop atmospheric testing go back twenty years and it is undeniable that public opinion was a major factor in securing the signing of the Partial Test Ban Treaty in 1963 by all nuclear and potentially nuclear powers except France and China. Since then signatories have been restricted to underground testing. Protests against the French activities were relatively insignificant until about a year ago, largely no doubt because the French test site is remote from large centres of population.

It was last summer's series, announced in advance, which drew the sharp criticism, at both national and personal levels. As it turned out that the devices were modest but in expectation of megaton tests several countries raised objections, of which the most interesting and substantial were those of Australia and New Zealand who proceeded against France in the International Court of Justice.

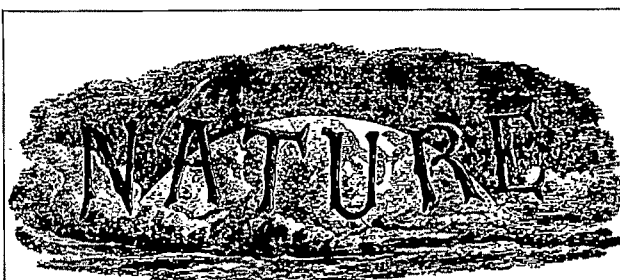
The International Court was told that tests violated, among other things, the rights of Australian and New Zealand people to be free from radioactive fall-out and to have free passage on the high seas. It was asked to order the French to desist from testing. The French replied that the court was not competent since the matter was one of national defence and France had already declared such matters to be beyond the court's jurisdiction. The French government was not represented at the hearings and when the court concluded, by a vote of eight to six, that "the French government should avoid nuclear tests causing the deposit of radioactive fall out" France chose to ignore the order. The French government prepared a White Paper justifying its stance legally and scientifically in strong terms.

The danger with questions of national policy is that they are not easily broken down into neat compartments. Few actions can be at the same time politically acceptable, legal, moral and safe. Furthermore, how safe is safe? If the French action is dealt with on grounds of legality and harm to the environment, the outcome will be muddy. As it was, the court decision was by a vote of eight to six and French scientists could be found to match Australian scientists on the safety issue. Those who seek a clear resolution of this conflict by law or scientific discussion are pushing in the wrong direction. Nor is the French government on any firmer ground in saying that international hostility comes from a political desire to impede French defence policy and spite French independence. Many of the most vocal countries have little concern for French defence policy and many of those who do worry about it registered no protest. The issue at stake is not whether the possession of nuclear weapons is justified or sensible. It is, in the final analysis, whether firing nuclear explosions in the atmosphere is a civilised or moral thing to do in 1974.

It is very difficult to argue such an issue through reasoned debate. Nevertheless, it is possible for a national consensus to be reached and it is incumbent on governments which sense such a feeling to act appropriately. Labour in opposition expressed disapproval.

As for the French, the signs are that things are changing under increasing moral pressures. There are ample rumours that an underground test site is being developed at Mururoa atoll, maybe to be ready by 1975.

100 years ago



Note on apparently useless Colouring in the Flowers of a Fumitory (Fumaria capreolata var. pallidiflora, F. pallidiflora Jord.)

I observe that in this plant at Mentone the flowers attain their brightest colouring after the ovaries are set, and when fertilisation is no longer necessary, or indeed possible. During the period previous to impregnation, the flowers are pale and nearly white, and the pedicels erect or horizontal; afterwards they become pink, or even crimson, and the pedicels are recurved, and the colour of the petals, which retain their form and position until the ovary has nearly attained its full size, intensifies with the lapse of time.

If the reverse had been the case there is little doubt that we should have regarded the bright colouring as specially adapted to attract insects, and as existing for that purpose, insects being, according to Prof. F. Hildebrand,* important agents in the fertilisation of fumitories; but here, as the brighter flowers are those which no longer need or are capable of profiting by the interference of insects, this explanation ceases to be possible.

This little fact, therefore, would seem to be one which might be classed with those which teach us that, side by side with the developments and modifications which are plainly beneficial to the organism of which they form a part, there are others, which, as far as we can see, are neither useful nor harmful to their possessor, though they may, and frequently do, supply features which especially attract our attention and admiration.

J. TRAHERNE MOGGRIDGE

From *Nature*, 9, 423, April 2, 1874.

Cancer and leprosy in South Asia

Professor E. J. Ambrose, of the Chester Beatty Research Institute, London, describes in particular the work being carried out on leprosy and cancer in Bombay.

MOST of the major health problems in South Asia are due to infectious diseases into which research is highly advanced, and the control of the diseases has mainly become a public health problem. There are two exceptions to this—cancer and leprosy. Cancer is a disease which, with the increase in life expectancy, affects a progressively increasing proportion of the population. It is already the fourth ranking killer among the over-45s in Bombay. Cancer research is now an intensive worldwide activity but there are many unsolved problems; as they occur in South Asia these have a different emphasis from those of western countries, for example the disease may be very advanced before the patient reaches a specialist centre requiring more radical forms of treatment and therapy, and the patient may be more sensitive to drugs (because of dietary factors) than is usually the case in the West; also, cancers at certain sites (oral and pharyngeal cancer from chewing habits, for example) are more prevalent.

Tata Memorial Hospital

The Tata Memorial Hospital in Bombay has for many years been engaged in the specialised treatment of cancer, with patients from all parts of India and other countries coming for treatment. In 1956 a new research institute, the Indian Cancer Research Centre, was built under the guidance of Professor V. R. Khonolkar, its first Director. Following his retirement in 1963 the hospital and centre became amalgamated, under the Department of Atomic Energy, as the Tata Memorial Centre under the directorship of Dr J. C. Paymaster. Particularly important during this time were the developments in cancer epidemiology. Dr D. J. Jussawalla became director in 1973; he too is interested in this field.

Cancer

The Indian communities, with their extremely rigid marital customs, still provide a unique field for the study of cancer epidemiology, but intermarriage may destroy all these possibilities within the next 50 years. Carefully planned activities are now, or soon will be, in operation; already a study is in progress in a rural area (Colaba) and others are in prospect. The chewing of Pan with tobacco is a major factor in oral cancer, but chewing, smoking, the bidi and dietary factors also lead to a high incidence in the pharynx, oesophagus and upper intestinal tract generally. The most valuable information is likely to be obtained from comparative studies of Indian migrant populations who have acquired new habits, particularly in Africa. Breast cancer is also common in India and epidemiological studies of the incidence in Parsi (late marriages) and Hindu women (who have early marriages) show a higher incidence among Parsis.

These epidemiological studies are being backed by collateral studies in carcinogenesis at similar sites. Of particular interest have been the studies in breast cancer. An inbred strain of mice (ICRC strain) has been developed which shows a high incidence of breast cancer and of leukaemia. Interesting relationships between B and C particles and the role of hormones in cancer incidence have been worked out with these mice. Ultrastructure studies are proceeding in human breast cancer and in the investigation of common base sequences in B particles and in the nuclear DNA of breast cancer patients.

A second major research theme at the centre is immunobiology. Studies in oral cancer have already shown evidence

for cellular immune response, particularly in patients with oral tumours subjected to electrical cauterisation.

The third theme now being embarked on is combination therapy, with special reference to cancers common in India. Under Dr Jussawalla, important advances have been made at the Centre in treating patients with a combination of chemotherapy, radiotherapy and surgery for various types of cancer. The centre has potential for developing new drugs from extracts of Indian natural products. A screening programme for plant products based on National Institutes of Health procedures has been set up using P388 and L1210 mouse tumours. Several active plant products have been isolated. The value of such studies probably lies in adding to the repertoire of drugs available for combination therapy. Systematic experimental work is being carried out on the characterisation of relevant human tumours. These include drug assays by organ or monolayer culture, basic cytology and, in particular, cell surface studies. An important advance has been made in isolating from cobra venom pure proteins which are not neurotoxins but which combine with specific sites in the surfaces of cells including tumour cells. Such agents are likely to be of value in the cytodagnosis of human cancers. Of very topical interest is the work on nuclear magnetic resonance. This approach shows promise as a diagnostic aid based on the spin lattice relaxation time of cell water.

Leprosy

Leprosy is in some ways a more pressing problem in South Asia than cancer. The number of leprosy cases in these areas totals several million and is on the increase. Theoretically, leprosy could be prevented by an intensive public health programme but in practice it may be many years before these programmes lead to a reduction in the number of cases, particularly in view of the population explosion. Dapsone treatment is effective, particularly in early cases, but requires daily doses for 5–10 years or even a lifetime in lepromatous cases. Infection continues to spread in the population at large despite fine work by devoted workers engaged in the care of leprosy patients.

Professor Antia, at the Tata Department of Plastic Surgery, has for a number of years been engaged in plastic surgery of leprosy patients. This is extremely important for rehabilitation, leprosy being almost as much a social problem (with its stigma) as a medical problem. He has also built up with the support of industrialists training centres where leprosy patients can learn new crafts and engage in active work in small factories. But the total number of workers in the field is still small.

A team is being built up for various studies, including pathology of the most suitable sites for biopsy of leprosy patients, the investigation of the localisation of bacillae in nerve cells and their effects on nerve conduction velocity. Teams work on the cell biology of leprosy, including the intracellular culture of the organism, and on the use of the mouse footpad. Developments in the general field of microbiology and cell biology may well prove valuable in the future for leprosy. Techniques for the rapid assessment of the viability and growth of the bacillae may lead to a deeper understanding of the metabolic requirements of the organism and hence to systematic research in leprosy chemotherapy (for example DOPA itself might prove to be a specific metabolite for *Mycobacterium leprae* (Prabarkharan)) and the problem of treating intracellular bacillae as well as extra-cellular bacillae may now be investigated in living systems by autoradiographic methods so that the systematic study of human biopsy specimens is possible.

This account refers specifically to work in Bombay, with which I am familiar. In other parts of South Asia also, dedicated workers are actually engaged in the treatment of these two diseases and in research. The need is great because a large fraction of the world population lives in this region.

international news



The Pasteur Institute — independent but fading fast? Picture by Robin Laurance

AFTER nearly 90 years of unparalleled contributions to medical science, the Pasteur Institute in Paris is battling against a financial crisis and is in danger of closing. Set up with private money in 1888 for Pasteur to treat his rabies patients, the institute has remained stubbornly independent ever since. Over the years its researchers have won no less than eight Nobel prizes for medicine and have developed sera and vaccines for rabies, yellow fever, diphtheria, tetanus, tuberculosis and influenza. But despite this, and because of the escalating costs of research, the institute is running at a 15% deficit on its annual budget and observers say it could close down within five years.

The job of giving it a new lease of life falls largely on its director Jacques Monod, a musician turned biologist who decided there were "better chances for second rate biologists than for second rate orchestral conductors." But far from being second rate, Monod shared with two Pasteur colleagues a Nobel prize for medicine in 1965 for fundamental work on genetics; and in 1970 published *Chance and Necessity*, a philosophical treatise on the mechanics of inheritance, which was a best seller in France running second only to *Love Story*.

The headquarters of the institute in the Rue du Docteur Roux on Paris's Left Bank includes a 120-bed hospital for infectious diseases, a comprehensive science library and a total research staff of 1,150 including 300 postgraduate

Guiding the Pasteur through financial troubles

Robin Laurance, Paris

trainees. High specialisation is the keynote of the institute since it is orientated largely towards microbiology. Fifty laboratories provide facilities for basic and applied research into bacteriology, virology, physiopathology, immunology, molecular biology and other related studies.

Monod's office looks across to the original building where Pasteur lived and worked, and where doctors still treat rabies today in Pasteur's small book-lined consulting room. "One of our problems", says Monod, "is that we still live in the past. Members of the old institute fail to realise that we cannot maintain the position and style the institute enjoyed in the early part of the century."

One of the most severe financial handicaps has been the absence—until 10 years ago—of any patents on the institute's products. "If we had been collecting royalties all these years", says

Monod, "there would be no financial crisis." Now, Monod has to enforce the patent policy "even more ferociously." He has asked his colleagues not to publish a single word about new discoveries until a patent has been applied for. And it is a rule that non-commercially-minded scientists find hard to accept.

But even with royalties and licence fees, the institute's income was still not going to match its research needs. So last year Monod formed the production and sales side into a high powered commercially orientated organisation called Institut Pasteur Production (IPP) with all the shares held by the Pasteur Foundation. Monod became President and he spent nine months finding the right men to run it for him. He brought in Nestlé's head of home marketing as Vice-president and Michelin's sales chief to run the marketing division.

Monod is bitterly opposed to any suggestion of a government takeover and even less willing to see the institute absorbed into the university system. And he insists that he is ethically justified in enjoying the freedom from state or capitalist control. "Of course no French government could afford to watch the institute disappear because it carries such prestige—it's a national emblem. But if it did have to step in it would appoint the executive and effectively take control. Everything would get bogged down with typical French bureaucracy—the plague of this country of ours—and Pasteur would lose a great deal. And the universities are so badly

run, it would be even worse to come under their control." Monod has no hesitation in saying he would resign on the spot in the event of either fate.

A fund-raising campaign last year raised £2 million, and IPP's first year showed promising results with sales totalling £8 million, due largely to a new 'flu vaccine which looks like being more effective than anything else on the market. Two million shots have been sold since last October but it is still not available in the United Kingdom or in the United States as it has yet to satisfy the drug control authorities.

"I think the authorities have gone too far", says Monod. "The thalidomide disaster has made them overcautious and companies bigger than our own IPP are facing closure because the exhaustive trials are simply costing them too much. When I'm in a good mood, I think of the whole thing as a kind of sport. Maybe the odds at 2:1 in favour, and that makes it a game worth playing."

The institute's latest discovery, about to undergo clinical tests, is a vaccine which will give the body increased resistance to all bacterial and viral diseases. If proved successful it could be the discovery of the decade. But with IPP's distributor in the United Kingdom forced to offer it at such low prices for vaccines to compete favourably on the British market, it too may never be used in Britain.

"It is a mistake", points out Monod, "to believe that we have come to the end of vaccine discovery." He says that whooping cough vaccine is still far from satisfactory and that vaccines against viral hepatitis and against gonorrhoea are urgently needed. And it is these fields, and in immunology as a whole, that Monod believes the institute's researchers can, given the chance, make vital contributions. "But there's no doubt", he warns, "that there is still a very serious danger of the institute closing. If IPP is given time to grow—and we must treble the present sales—then we'll be all right. It all depends on how long that takes."

AEC case for fast breeders

Colin Norman, Washington

IN June last year, the US Court of Appeals in Washington dealt a potentially severe blow to the United States fast breeder reactor programme—the great white hope for meeting the country's energy needs by the end of this century. The court ordered the US Atomic Energy Commission (AEC) to obey the laws of the land by publishing a comprehensive analysis of the combined environmental effects of several hundred breeder reactors scattered throughout the United States. The AEC has now produced a massive draft report which predictably argues that the reactor will be environmentally acceptable, safe and desperately needed.

The report, which runs to some 2,000 pages, is now doing the rounds of other government agencies, public interest groups and environmentalist organisations, and it will be the subject of a public hearing on April 24. Not surprisingly, it is generating a good deal of heat in the long and bitter battle between the Atomic Energy Commission and its critics over the safety and acceptability of nuclear power.

The objective of the court suit, which was brought by the Scientists' Institute for Public Information, was to force public discussion of the dangers and environmental consequences of the entire commercial breeder reactor programme, before development had progressed so far that it would be difficult to halt. The critics reckoned that if the huge scale of the proposed programme, together with the dangers inherent in fuel processing and waste disposal, could be brought into the public spotlight, the case against the breeder would be compelling.

But the AEC, helped by the energy crisis in general and by the Arab oil boycott in particular, has turned the argument about the size of the programme around and thrown it back in

the faces of its critics. The agency argues, in short, that the breeder reactor will offset United States dependence on imported fuel, that it provides the surest and most acceptable means of meeting the predicted demand for energy in the 1990s and beyond, and that to abandon breeder reactor development now could put the United States in the position of relying on foreign breeder technology at some time in the future.

First, the scale of the programme. The AEC's report indicates that if all goes according to plan, the first commercial breeder reactor will start operating in 1987 and by the year 2000, 400 individual plants, each capable of producing 1,000 MW of electricity will be at work in the United States. They would be producing between them as much electricity as the entire United States electric power industry generated last year. By the year 2020, as much as 2.2 million MW could be generated by fast breeder reactors.

The AEC argues that some other means of providing that energy must be found if the breeder reactor programme is halted and that there is nothing on the horizon which is either capable of filling the gap or technologically proven. To which the critics reply that if the money to be spent on breeder reactors were put into other energy systems, such as solar power and geothermal energy, then they could be developed to the point where they would be capable of meeting energy demands. The AEC replies that if it turns out that the concepts, promising as they are, cannot be turned into energy in time, the United States would find itself in an energy crunch by the end of this century.

What particularly worries opponents of the breeder reactor is the amount of plutonium which will be produced by a string of reactors operating around the country. The advantage of the breeder over conventional nuclear power plants is that it can be fueled on nonfissionable uranium which cannot be used in the so-called first generation reactors. The uranium is converted during the reactor operation into plutonium, which can

Dounreay — Britain's first fast breeder station, on line without objections



then be used to fuel the reactor on another operating cycle—in other words, the reactor breeds its own fuel.

The advantage is that the breeder reactor does not require additional uranium to be mined, which is fortunate because uranium reserves in the United States are estimated to last for only another 25–30 years. But the problem is that plutonium is highly toxic, its radioactivity decays extremely slowly and it can be turned relatively easily into the heart of a crude atomic weapon. Opponents of the breeder programme have therefore concentrated their attacks on the health and national security hazards of plutonium. Clearly, the larger the commercial breeder programme, the more plutonium will be produced, and the greater the perceived hazards.

The AEC analysis states, however, that strict safeguards are enforced at all facilities which handle plutonium, to prevent any of it from falling into the hands of people looking for a nuclear weapon. Unfortunately, however, a study conducted last year by the General Accounting Office (GAO) concluded that the safeguards at some installations would scarcely deter an amateur burglar, let alone a well planned theft. Although the AEC maintains that it has stepped up its safeguards in the light of the GAO's findings, the public relations aspects of the matter are difficult to overcome.

As for the health risks, the AEC's draft report concludes that the entire breeder reactor programme is likely to cause less than 2.2 cancer deaths in the United States over the whole lifetime of the reactors, fuel fabrication plants and waste disposal facilities. But that conclusion was challenged even before it appeared in print by two nuclear energy specialists working for an environmental organisation called the Natural Resources Defense Council and it is likely to develop into a bitter wrangle.

The two critics of the AEC's analysis, Dr Arthur Tamplin, an ex-AEC employee and long a thorn in the agency's flesh, and Dr Thomas Cochran, have challenged the basis of the nuclear industry's estimates of the potential damage caused to the lungs by particles of plutonium. They have argued, in short, that it is unrealistic to base safety standards on the likely dose of radioactivity to the entire lung from tiny particles of plutonium. A single particle is likely to give a dose of radioactivity to the whole lung of about 0.003 rem per year but Tamplin and Cochran argue that the tissue immediately surrounding a particle of plutonium will get a dose of radioactivity as high as 4,000 rem per year. Therefore, they argue, the AEC should reduce the amount of plutonium allowed

to be released into the atmosphere by a factor of 115,000.

Those two points—the potential for diverting plutonium into the hands of somebody capable of producing a crude bomb and the health risks from plutonium released into the atmosphere—are thus likely to form the centrepiece of the public hearings next month. But environmental causes have been taking a battering in the past few months in the United States, as public attention has swung away from the environmental crisis to the more obvious effects of the energy crisis, and the AEC can be expected to play the breeder reactor's promise of supplying 2.2 million MW of electricity for all it's worth.

It is, perhaps, worth noting that earlier this month, the British 250 MW Prototype Fast Reactor at Dounreay became critical, and the fast breeder reactor programme is proceeding in Britain relatively unencumbered by requirements for public discussion of risks and benefits.

Nuclear inquiry

WITHIN weeks of the Dounreay Prototype Reactor going critical, and while the choice of Britain's next nuclear reactor system hangs in the balance, the Royal Commission on Environmental Pollution has announced its decision to investigate standards of nuclear safety in the country. The Commission on Environmental Pollution is a standing commission, recently reconstituted under the chairmanship of Sir Brian Flowers, and the choice of subject for its first inquiry is thought to have been decided by the long term implications of a switch from conventional to nuclear power generation rather than by the immediate crisis of consciences over hardware selection.

Sir Brian has taken pains to point out that members of the commission "do not feel able to involve themselves in highly technical questions of reactor design in relation to safety, or with comparisons between one type of reactor and another". Nevertheless, in the next breath he added that in the general grounds of concern about environmental hazards "in a choice between reactor systems great and perhaps overriding weight should be given at the present time to the issue of safety". The commission will also examine the problems of radioactive waste disposal and the transport and storage of nuclear materials, particularly plutonium.

Business report

Roger Woodham

ALBRIGHT and Wilson, the chemical company, has had to reduce the book value of this ill-fated phosphorus plant at Long Harbour, Newfoundland, because of continuing production difficulties. In 1973 the plant, designed to produce 72,000 tons of phosphorus a year, only had an output of 40,000 tons, which, at that, was an improvement on the situation in 1971 and 1972.

The Long Harbour plant is one of only four of its size in the world—it consumes 140 MW of electricity—and Albright and Wilson admitted in 1971 that the two manufacturers using a comparable electrode arrangement in the furnace had experienced and overcome similar difficulties—broken electrodes. Nonetheless these problems, which have necessitated several major shutdowns, do not seem to have been sorted out at Long Harbour and the company is throwing in at least part of the towel by revaluing the plant from £19.9 million to £11.2 million.

In spite of these upsets Albright and Wilson managed to notch up an increase in profits from £2.75 million to £7.47 million, just short of its 1964 best of £7.57 million.

With prices of raw materials as they are, however, the company's future fortunes are going to depend on how one of its major customers, the farmer, reacts to the prospect of higher phosphate prices.

● The prices in Britain of scientific instruments are by no means all susceptible to the ravages of inflation, although the cost of an instrument that pushes a technique to the limit possible at a given time does jump ahead in leaps and bounds.

Instrument price trends

	Routine	Research
NMR	£10,000 (£10,000)	£40,000 (£80,000+)
IR	£1,410 (£1,420)	£8,000 (£20,000)
UV	£700 (£750)	£5,000 (£8,000)
GC	£600 (£1,000)	£2,000 (£3,000)

Broadly speaking, the prices of routine instruments that have the same capabilities as their counterparts ten years ago, say, have marked time and in real terms have fallen. The reason is that companies have made use of new technology to simplify and cheapen the manufacturing process. With really high performance equipment, on the other hand, the manufacturer is involved in much larger development costs and is much more sensitive to the effects of wage inflation. Top notch instruments

are thus much more expensive now than they were ten years ago, a situation which is often described in terms of a 'sophistication factor'. In the case of imported instruments, which account for a greater proportion of the research type than the routine type in Britain, the customer also, of course, has to contend with price changes introduced by fluctuating exchange rates.

The table shows the state of affairs for nuclear magnetic resonance (NMR), infrared (IR) and ultraviolet (UV) spectroscopy and for gas chromatography (GC). The prices in brackets are the prices today and the others are the prices ten years ago.

Oil on troubled waters

A SYMPOSIUM on hydrocarbons in the marine environment has been postponed by the Ministry of Agriculture because they do not think the meeting is necessary. The organisers, the Torry Research Institute and the Marine Research Station at Aberdeen, planned to discuss the effects of oil pollution at Aberdeen in May. About 100 scientists said they would attend, but they are now being told that the symposium has been postponed. Apparently, somebody who should have been asked wasn't. The Ministry of Agriculture's Administrative Officer, Mr W. R. Small, explained that the symposium is not necessary because there are "six or seven" other symposia going on which deal with the same, or allied, subjects. "This should never have been mooted in the first place without the right people being asked",

he thought. "There is simply no unanimity about the urgency of the symposium at this stage. There is a limited corpus of scientists scurrying around these meetings, and at this moment the ministry cannot afford to have its resources spent unnecessarily. You know, it's like a Beau Geste fort, with the same men appearing at different battlements all the time."

A corollary to this spirited assessment of the marine biologist's round is that the men who appear at the battlements on home ground tend to include a fair proportion of junior scientists who are actually engaged in research in the field. The overseas junkets are sometimes made up of more senior men who count foreign travel among their hobbies. The Aberdeen cancellation note points out that the symposium would have clashed with a similar meeting in Maryland—a conjunction which might have caused a crisis of conscience among the senior men, but would not have presented a problem to the junior workers who would have been glad of the chance to talk about pollution problems on their own doorsteps.

Furthermore, Aberdeen is not doing at all badly for profits from services related to the offshore oil industry at the moment and some people would not really be interested to hear about hydrocarbon pollution if oil ran ankle deep in the streets. The Scottish press is keen to pick on stories involving damage to the environment and would almost certainly have been looking for pickings at the Aberdeen symposium. There was a chance that the benefactions of the oil industry would be called into question. This has now been avoided.

More planning problems at Loch Carron

THE public inquiry into setting up deep-sea oil platform construction sites at Drumbuie, Loch Carron on the west coast of Scotland opened again on March 18 after being adjourned for several weeks, but was immediately adjourned again at the request of the National Trust for Scotland, the main objector. The trust was opposed to an entirely unexpected environmental report being placed as evidence.

The trust owns the land on which the platforms will be built if the Drumbuie site is approved for development. They were objecting to a surprise environmental report commissioned by the Scottish Development Department from Sphere Environment Consultants of London. This report considered several other possible sites in the area. None of the objectors had been told that the report was in preparation and their counsel successfully moved for an adjournment until April 1.

The National Trust and the other ob-

jectors had finished giving their evidence against the development when the inquiry was adjourned in February, and this new development will considerably prolong matters. The environmental report and an expected consulting engineer's report, commissioned by the Department of Energy, both come out in favour of Drumbuie as opposed to other sites in the area but give alternatives if the National Trust land does not become available. As the proposed bill to nationalise land needed for oil platform construction is not going ahead under the new Labour government, a special bill in Parliament will be needed to use the trust's land.

Sphere suggested a site on the Isle of Skye near Broadford Bay as their second choice. On the other hand the consulting engineer's report gives a site on Loch Kishorn, near Carron, as the second choice and Broadford Bay as the third.

Australian science news

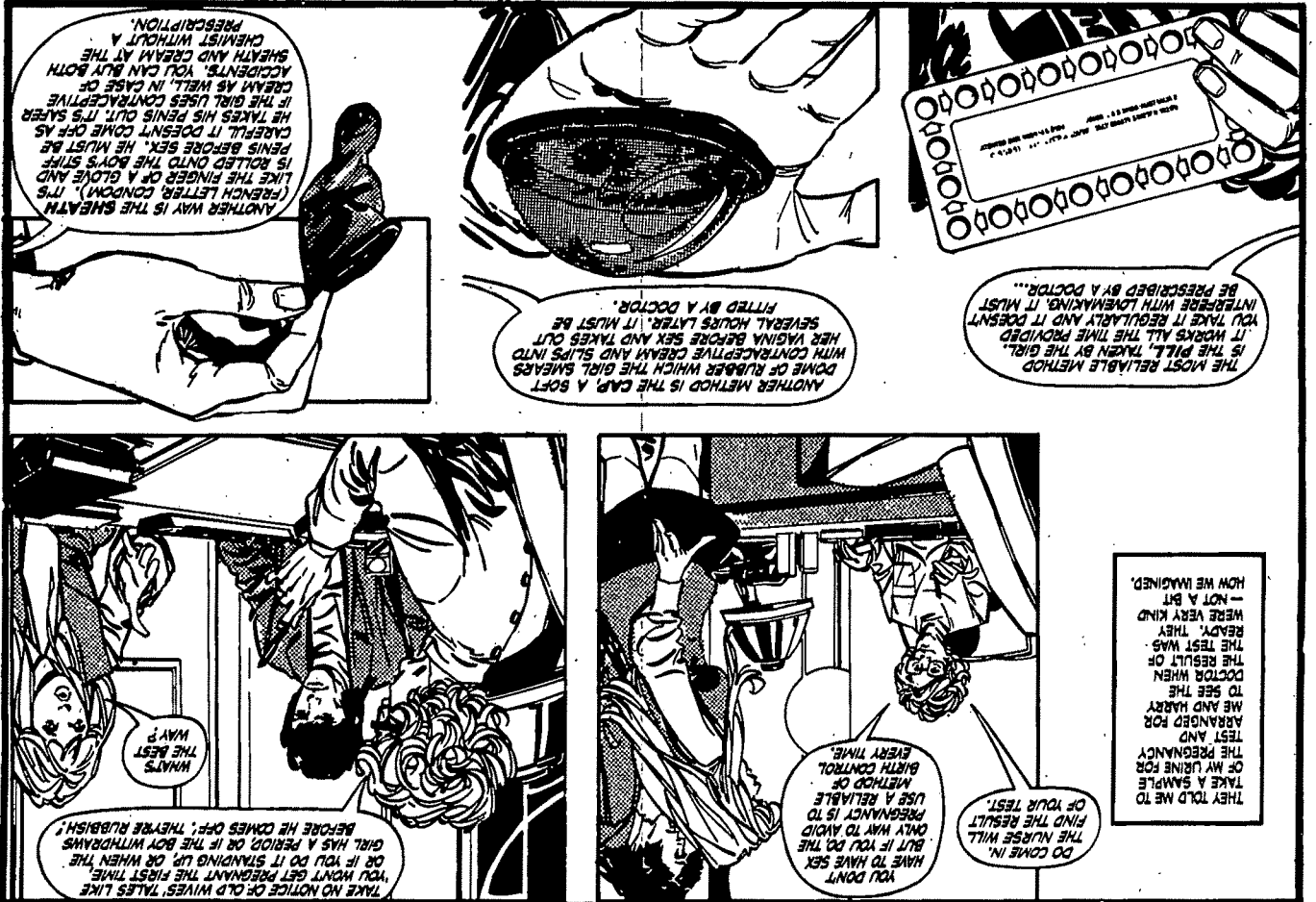
Peter Pockley, Sydney

IN a speech to the Science and Industry Forum of the Australian Academy of Science in February, Dr Moss Cass, the Minister for the Environment and Conservation, announced the formation of a new Bureau of Environmental Studies within his department. In doing so, Dr Cass has rejected alternative proposals for establishing a statutory institute, or for strengthening the existing responsibility for some environmental research within the Commonwealth Scientific and Industrial Research Organisation (CSIRO), which in any case comes under the Minister for Science, thus revealing the old problem of duplication where departments overlap.

The Bureau of Environmental Studies will have three main areas of responsibility. First, it will survey, assess and integrate existing studies done by other groups. Second, it will develop programmes to fill identifiable gaps. A third group in the bureau will try its hand at environmental 'futurology', with the aim of anticipating the problems of 20 to 30 years ahead. Here we see the strong strand of anti-industrial growth and humanism in Dr Cass's philosophy, for this group will be charged with developing some kind of human progress index. For this, Dr Cass suggests the term 'gross domestic welfare', which he hopes could be derived from a combination of measures of environmental quality, social indicators and productive capacity in both goods and services. Recruitment for the bureau's staff will start shortly. It looks as though they will need to be in the market for social philosophers as much as ecologists.

● In a further move indicating the diplomatic initiatives between the Australian Labor government and the communist world, the first exchange of visits between Australian and Chinese scientists was announced recently. A party of nine Australians started an 18-day visit to Canton, Peking, Shanghai and other centres on March 18. The party of four representatives of the Australian Academy of Science, four from the Australian National University (ANU), and one Asian scholar as interpreter will be led jointly by Professor Sir Rutherford Robertson, President of the Academy, and Dr R. M. Williams, Vice-Chancellor of ANU. The inclusion of an interpreter chosen by Australia is seen as a significant advantage for the party; he is Professor Liu Ts-un-yan, Head of the Department of Chinese at the ANU.

The scientific interests of the Australians include astronomy, spectroscopy, plant physiology, genetics, mathematical



In Britain the Family Planning Association (FPA) has taken over the production of a comic about birth control methods, called *Too Great a Risk*, which was the brainchild of the FPA's South-west London branch. The headquarters of the FPA took control of production and distribution because the comic proved so successful on a local level: since its launch in March last year 70,000 copies of *Too Great a Risk* have been supplied to school and college teachers, youth

statistics, classical thermodynamics, nature conservation, biomedical sciences and civil navigational aids. The party will officially be guests of the Academia Sinica, who will sponsor a return visit of Chinese scientists to Australia later in the year.

● Only a few hours before rushing back to Britain to preside over the general election, Queen Elizabeth performed her first major constitutional function in her newly designated role as 'Queen of Australia'. This was the formal opening of the 28th Federal Parliament in Canberra. Despite the governing Labor leaders' avowed preference for a republic in Australia in due time, this occasion was marked by as much ceremony and circumstance as in the Queen's speech at the opening of Parliament on February 28 revealed, in tersely abbreviated terms, a massive programme of legislation which Prime Minister Whitlam's government hopes to push through in its second year in

office. (Some of it will truly require much hoping and pushing because the government faces a hostile opposition in the Senate where the opposition parties hold a firm majority—a Senate election due in the middle of this year is unlikely to change the balance of power in this upper house.)

Science *per se* rated a mere six words, namely "A science council is being planned". This passing mention con-firmed how tentative are the current plans of the Minister for Science, Mr Bill Morrison, for replacing the former Advisory Committee for Science and Technology which he axed a year ago. Mr Morrison has said that legislative action is needed to establish the proposed Australian Science Council on a proper footing but hopes are now dim-pling that his portfolio has enough political pull to get some priority for science affairs on the legislative list. It now looks unlikely that a Science Coun-cil could be formed before next year, the last scheduled year of Labor's

three-year term.

Previous speculation that Mr Morris-son would be promoted out of the science portfolio is now dead. He has shed all but residual responsibility for Papua New Guinea affairs and there is a fair flow of publicity over the min-ister's name, relating mostly to admin-istrative changes within the minister's responsibility. But the politics of science in Australia are rating no mention at all from the news journalists, the com-mentators and, worse, from the politi-cians themselves.

● Postscript to our news (Nature, 247, 500, 1974) of the appointment of Pro-fessor E. Joseph Wampler as Director of the Anglo-Australian Telescope. His salary has now been disclosed as \$A23,000. This is \$A3,850 more than that of the new Director of the Austra-lian Institute of Marine Science (which is the equivalent to the salary of a full professor) and is within the range of salaries paid to Chiefs of CSIRO Divisions.

correspondence

Academics in Chile

SIR,—The international scientific community must be aware of the dramatic condition of the Chilean academic community. The military authorities who have taken over the power in all Chilean universities issued new rules stipulating that:

"Any nomination or contract must from now on be considered strictly provisional. The Special Commission [installed by the military rector] will propose renewals or cancellations of such contracts in each particular case.

All students must apply for reinscription . . . The Special Commission will decide on the opportunity of accepting or refusing such applications.

Any professor, administrative employee, technician or student suspected by civil or military courts will be immediately suspended . . . Any one of them being sentenced will be dismissed; students will be permanently expelled from the Universities.

The same action will be taken towards those having answered citations from the courts . . ."

This quotation is taken from an official statement issued by the new authorities in charge of one Chilean university on September 28, 1973. Analogous decisions have been made in all universities, including the Universidad Catolica de Chile. As a consequence of this, many professors or academic staff have lost their jobs. In the most favourable cases (such as that of professors on official leave or absence abroad), scientists "have been granted permission to present their resignation" (usually with retroactive effect). Many have been arrested, on an arbitrary charge or no charge at all, usually after anonymous denunciations of neighbours or colleagues. Many of them are still in prison and an official International Lawyer's Commission has presented evidence for many cases of physical torture. In La Serena, professors against whom no special charge could be found have been executed in spite of having been condemned to minor sentences (less than two months of imprisonment).

Several other members of the academic community have been executed without being permitted to present their defence.

In various universities, departments like geography, sociology, economics and biophysics have been completely dismantled.

These facts must be publicised and

scientists all over the world should press governments and international organisations in order to prevent further repressive actions. In addition, there is an urgent need for help to academic people who already could, or will eventually be able to, leave the country. We have on record many excellent applications in all fields ranging from mathematics to social sciences.

All those able to offer laboratory space and positions, even on a temporary basis, should contact the Committee of Assistance to Chilean Scientists (CACS), by writing to M. Imbert, Collège de France, 75231 Paris-Cedex 5.

Yours faithfully,

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North Sea oil

SIR,—Although I agree with the opening paragraph of Eleanor Lawrence's article on the planning aspects of North Sea oil (*Nature*, 247, 416; 1974) I feel that the remainder of the article leaves much to be disputed.

What does Mrs Lawrence mean by 'getting the oil ashore as quickly as possible'? If she means seeing the oil flow as soon as possible then there is probably nobody against it. Already, nine platforms are in various stages of construction for the Auk, Beryl, Brent and Forties fields, and at least one or two other fields will be exploited using drilling rigs rather than production platforms. There is, therefore, no block to how quickly the first oil will come ashore. If, however, she means by 'the most extreme preservationists' those who are opposing the intended maximum rate of exploitation of this important resource (as is current policy) then she is referring to no splinter group of cranks. It includes Liberals, Scottish Nationalists and a good number of Socialists who have all committed themselves against seeing oil used in this way.

It is, I would agree, right to criticise present planning procedures because

they treat each separate proposal in isolation rather than in the context of a coordinated plan. This is plainly ridiculous. The advantage that the present system offers, however, is time to reflect. The technological situation in relation to oil extraction from the sea bed is changing so rapidly that there are real dangers that what seems essential today will be of historic interest in a couple of years' time. Thus although the Condeep gravity concrete structure is currently in favour (and if to be built in Britain, probably needs to be constructed at Loch Carron) this ignores the alternative deep-water platform designs already being offered by different companies at existing yards, ignores the recent innovations in 'floating' platforms announced recently and ignores the technology for seabed completions which it is expected will be erected for deep water cases within the next two years. It would be a tragedy if rushed decisions caused the destruction of a way of life at Loch Carron, especially if it were for only a short term return.

The real iniquity of the present system is that the objectors should be involved in costs of many thousands of pounds. This is not because of the law itself but because of government choice. Section 267, subsections 7 and 8 of the Town and Country Planning (Scotland) Act 1972 states clearly that costs may be awarded to the objectors but the Secretary of State for Scotland has consistently refused to consider doing this. Thus, although objectors must pay their costs from their earned and taxed incomes, development companies can offset their costs against profits before tax. At Dunnet Bay the objectors were faced with a bill of £3,000 while Chicago Bridge decided to go elsewhere. At Loch Broom, the Action Group incurred costs in setting up its case against the proposed development at Ullapool before Mowlem discovered that the site was unsuitable anyway. Meanwhile the taxpayer contributed to an environmental impact survey that was never needed. The main objectors at Drumbuie will be faced with bills of tens of thousands of pounds. There is a very clear case indeed, in the light of the special circumstances that oil developments present, for the proposing companies to be held responsible for costs of genuinely concerned objectors.

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news and views

Genetic factors in hepatitis

ALTHOUGH much has been written about the possible mechanisms leading to the establishment of the chronic carrier state of hepatitis B antigen and the remarkable geographical variation in the prevalence of such carriers, which may number in some areas many hundreds of thousands, the genetic hypothesis promulgated by Blumberg and associates (*Proc. natn. Acad. Sci. U.S.A.*, **62**, 1108; 1969) remains a contentious issue among students of viral hepatitis.

Examples of inherited susceptibility to infection have been described in several animal species including man and it is recognised, of course, that many other factors in the environment such as sex, age at exposure, state of nutrition, immunological factors, socio-economic circumstances and so on also affect susceptibility and resistance to infection. The discovery of hepatitis B antigen resulted from the interest of Blumberg and associates in inherited polymorphisms. Family studies carried out in the islands of Cebu and Bougainville in the Pacific, and on a more limited scale elsewhere, seemed to be consistent with the hypothesis that a gene designated *Au¹* is responsible for persistence of hepatitis B antigen once infection with hepatitis B virus has occurred. This gene is considered to be common in tropical areas but rare in temperate zones. Individuals homozygous for this gene (phenotype *Au¹/Au¹*) would have persistent antigen without overt manifestation of hepatitis, although they may remain as carriers of the associated infectious agent, whereas the heterozygotes (phenotype *Au¹/Au*) and individuals without the gene (*Au⁰/Au⁰*) would not remain as carriers. Persistence of hepatitis B antigen in certain other diseases such as leukaemia, lepromatous leprosy and Down's syndrome (mongolism) is believed to be of a different nature, namely an inherited susceptibility to infection not only with hepatitis B but with the agents responsible for these other diseases (Blumberg, *Ann. N.Y. Acad. Sci.*, **197**, 152; 1972). This would explain the association of the antigen with other diseases in circumstances in which the individuals with the susceptibility gene were exposed to hepatitis B virus; and absence of the association when exposure did not occur.

Ceppellini and colleagues (Convegno Farmitalia, *Antigene Australia ed Epatite Virale*, Minerva Medica, Torino, 53; 1970) also studied the segregation of the postulated trait for persistence of hepatitis B antigen among 165 families in Sardinia. A similar conclusion on the presence of an autosomal recessive locus was reached, although with some distinct qualifications. First, there was a sharp decline in the carrier rate of the antigen after the age of 20, an observation which has also been reported from many other high prevalence areas. Second, all of the reported matings, excepting one family, were between parents where only one parent was antigen-positive or where both parents were negative. In the family in which the antigen was detected in both parents, only two of the seven children were also positive. This finding on its own would rule out a simple hypothesis of recessive inheritance with complete penetrance of the homozygous recessive genotype. Ceppellini considered this fact, but decided to disregard the critical mating.

Now Vyas has reported (*Nature*, **248**, 159; 1974) a second critical mating which also fails to support the postulated inherited susceptibility to persistence of hepatitis B antigen. A pedigree with antigen-carrying parents and their progeny of three children were tested for hepatitis B antigen and hepatitis B antibody by several methods including the most sensitive techniques currently available—radioimmunoassay and passive haemagglutination. The antigen was not found by repeated testing of the serum of the three children. Hepatitis B antibody was, however, detected in the serum of the eldest son, aged 10 years, and so provides unequivocal evidence of past exposure to the antigen resulting in a normal immune response to infection. This is crucial evidence against susceptibility to the chronic carrier state inherited as a simple autosomal recessive trait.

Lederberg (*Hepatitis and Blood Transfusion*, edit. by Vyas, Perkins and Schmid, Grune and Stratton, New York, 89; 1972) previously summarised the position by stressing the alternative hypothesis to the genetic factor—that familial clustering may be entirely a function of an increased opportunity for environmental exposure to the virus. Furthermore, it stands to reason that if a parent is excreting the virus this will augment the chance that any of the offspring may acquire it, particularly if the mother is the transmitter.

Another aspect of the genetic basis of susceptibility to hepatitis is implicit in the finding that the frequency of certain genetic markers on the heavy chains of immunoglobulin G (Gm types) was always greater in multiply-transfused Italian patients with thalassaemia who were persistent carriers of hepatitis B antigen than in those with hepatitis B antibody (Blumberg and associates, *Nature*, **236**, 28; 1972). With respect to Gm types, individuals who are heterozygous for Gm factors are less likely to encounter Gm types different from their own and are therefore less likely to develop anti-Gm. Such persons when infected with hepatitis B virus, which incorporates certain host components in its protein coat, would be likely to develop persistent antigenaemia and minimal liver damage. On the other hand, individuals who are homozygous for Gm types (that is, they have fewer Gm types) are more likely to encounter Gm types different from their own and thus develop antibodies to them. Such persons, when infected with hepatitis B virus, would be more likely to have an acute infection and an increased probability of developing hepatitis B antibody. Schanfield and colleagues (*Nature new Biol.*, **243**, 81; 1973) examined for Gm allotypic markers samples from blood donors submitted routinely for testing for hepatitis B antigen in San Francisco and Minneapolis. The frequency of the various Gm phenotypes did not vary significantly among the Caucasian donors investigated according to the presence or absence of hepatitis B antigen or its antibody. Similarly, there was no significant excess of anti-Gm among individuals with hepatitis B antibody when compared with those with persistent hepatitis B antigenaemia. These findings are not in accord, at least in a normal adult population, with the hypothesis of a direct association between the polymorphisms of the Gm types and hepatitis B antigen.

It does not follow, of course, that the hypothesis of a genetic predisposition to hepatitis did not have a productive impact, since it did generate much discussion and considerable experimentation.

A. J. Z.

Blue-green algae as model organisms

SOME filamentous blue-green algae show a one-dimensional pattern of differentiated cells. In *Anabaena* and *Nostoc*, heterocysts arise from vegetative cells. Heterocysts are relatively large, rounded, refractile cells, which are believed to fix nitrogen. The transformation from vegetative cells to heterocysts by way of the intermediate proheterocysts can be stimulated by transferring algal filaments to a medium free of fixed nitrogen. The conditions required vary among the species; for example, in *N. muscorum* and *A. cylindrica* there is not a well-defined pattern of proheterocysts in a medium containing fixed nitrogen, although there may be in *A. catenula*.

Two separate problems have recently been addressed in the literature: first, how the pattern of heterocysts is controlled; and, second, what the function of heterocysts is and whether this can be correlated with the appearance of specific proteins during heterocyst differentiation.

Mitchison and Wilcox (*Nature*, **239**, 110-11; 1972) found that vegetative cells in *A. catenula* divide with a mean time of 14 h. The best defined event in the cycle is the formation of a septum between daughter cells which takes 1-2 h. The divisions are always asymmetric and follow a simple rule which had no exceptions in the 600 cases observed. If a cell arises as, say, the left daughter of a division then its left daughter is always the smaller. Furthermore, heterocysts (which do not divide) only develop from the small daughter cells, which themselves grow more slowly than the large daughters although both attain the same size before their next division. This provides a very simple example of determination following a cell lineage rule, as if the vegetative cells were behaving as a set of stem cells. Mitchison and Wilcox, however, say that they know of no other example in which this recursive rule is followed.

The spacing of heterocysts, although it depends partially on cell lineage, is finally determined by other factors, as most small daughter cells do not develop into heterocysts. Wilcox, Mitchison and Smith have examined these in detail (*J. Cell Sci.*, **12**, 707-723 and **13**, 637-649; 1973). In the first paper they define the pattern and make a model of the spacing mechanism; in the second they test the model experimentally, and report on the ultrastructural aspects of heterocyst differentiation.

In both species of *Anabaena* used the pattern of heterocysts is quite regular: in *A. cylindrica* there are 9.3 ± 2.8 vegetative cells between heterocysts (or preheterocysts) and in *A. catenula* there are 10.1 ± 2.5 . The simplest model to account for this was put forward by Fogg, who suggested that heterocysts produce a diffusible metabolite which inhibits further heterocyst differentiation. That is, there is a threshold concentration of inhibitor below which small daughter cells will differentiate into heterocysts. Wilcox *et al.*, however, feel that this model is too simple as they found that early differentiation towards heterocysts is reversible implying that there might be a competitive interaction as well.

In particular, the authors examined heterocysts that arose in the terminal intervals of filaments. In central intervals proheterocysts tend to appear exactly at their centre, but in the terminal intervals proheterocysts, and thus heterocysts, are displaced towards the end of the interval. On the simple threshold model one would expect to see heterocysts at the ends of terminal intervals, where inhibitor concentration would presumably be lowest. Wilcox *et al.* suggest instead, however, that an end behaves as though it were one half of a hypothetical interval consisting of the original terminal interval joined to its mirror image. That is, the end wall of the terminal cells acts as a reflecting barrier to inhibitor diffusion.

A terminal heterocyst would thus be equivalent to one half of an adjacent heterocyst pair, which almost never occurs. The addition of a competitive interaction to the simple threshold model could then account for the rarity of terminal heterocysts.

Wilcox *et al.* therefore made artificial ends by breaking *A. catenula* filaments in intervals where central heterocysts would normally be expected to arise. As expected, the developing heterocyst was displaced towards the break, producing a normal end interval within 8-12 h, a time too short for differential division to be important. They then, in *A. cylindrica*, made mirror-image heterocysts by breaking a cell close to a proheterocyst. As expected this led to regression of the proheterocysts, with a probability depending on the number of cells left intact between it and the break, although there are relatively few regressions when the cell next to a proheterocyst is broken. This may be because the proheterocyst forms a normal, permeable cell junction on the break side which could allow a leakage of inhibitor.

A further piece of evidence for compensation is that groups of proheterocysts may appear in *A. catenula*. All but in a group one regress, unless there are more than three cells in the group, when one heterocyst may differentiate at either end.

Wilcox *et al.* propose that even after a proheterocyst has begun to produce inhibitor it remains susceptible to its effects. When a threshold, which increases with developmental age, is exceeded, the cell regresses. The contribution of heterocysts to inhibitor production sum, so one can inhibit another. The most important feature, the competition, implies also that a heterocyst can inhibit its own development, as in regression after filament breakage.

In their second paper Wilcox *et al.* give further support to their model by investigating the conditions in which a heterocyst can inhibit itself. They used *A. catenula* and made filament fragments containing heterocysts at all stages of development with no, one, two or three neighbouring cells on each side. Regression frequency decreased both with developmental age and with number of neighbours. Moreover, heterocysts at the end of a fragment had a lower probability of regressing than heterocysts with a neighbour on each side, probably because inhibitor can leak through the heterocyst wall.

The competitive interaction which is enhanced by NH_4^+ can apparently be broken down by 7-azatryptophan (Mitchison and Wilcox, *Nature new Biol.*, **246**, 229-233; 1973). When it is added to the growth medium, at a concentration of $5 \times 10^{-6}\text{M}$ or greater, the spacing between heterocysts is reduced and multiple heterocysts may form. Alteration of the pattern begins within 10 h and is independent of concentration, although the final pattern is not. All the changes observed correspond to an increase of inhibitor level in their model, although there is no evidence to link the effect of 7-azatryptophan with any aspect of metabolism.

Fleming and Haselkorn (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2727-2731; 1973) separated heterocysts from vegetative cells, in *Nostoc muscorum*, by treatment of the filaments with lysozyme and then centrifugation. They found that the increase of nitrogenase activity, which occurs on transfer of filaments to a medium free of fixed nitrogen, parallels the increase of number of heterocysts formed, and that heterocysts synthesise many proteins not found in vegetative cells. Furthermore, they were able to isolate (on SDS gels) three proteins which bore a strong resemblance to the structural proteins of bacterial nitrogenase the induction of which was also related to the increase in nitrogenase activity.

The authors speculate that heterocyst differentiation begins when the concentration of NH_4^+ falls below a threshold and that products of nitrogen fixation in heterocysts repress the development of, and nitrogenase synthesis in, neighbouring vegetative cells. As the ability to inhibit

further heterocyst development is acquired before pro-heterocysts can fix nitrogen, the authors suggest that pro-heterocysts might be able to transport NH_4^+ to their neighbours. They also point out that heterocysts must become refractory to repression as they continue to synthesise the structural proteins of nitrogenase while fixing nitrogen. Although there are many ways in which induction and repression might be mediated, it is interesting that both groups working on this problem have been forced to models with very similar qualitative features.

The blue-green algae proved a very simple system for the study of patterned differentiation. It should be possible not only to discover the mechanism for the control of differentiation, but also to correlate differentiation with events at the molecular level, a normally elusive ideal for developmental biologists. *Anabaena* and *Nostoc* thus join *Hydra* and *Dictyostelium discoideum* as model organisms in which the outlines of a developmental control system are known, and which are simple enough that the details of differentiation during pattern formation can be followed.

From a Correspondent

A Chinese view of tectonics

PROFESSOR J. S. LEE was a Chinese earth scientist highly regarded both in China and the West, so the appearance of a long posthumous article by him entitled "Crustal Structure and Crustal Movement" in *Scientia Sinica* (16, 519-559; 1973) is an event of great interest. The paper was written in 1970, and so cannot claim to be an ideal representation of present-day Chinese thinking on the subject, but it is nevertheless a most fascinating insight into Chinese approaches to tectonics; the more so because Lee's conclusions, based primarily on Chinese evidence, are simultaneously near to and far from Western views.

In a subject as explosively developing as the earth sciences, it is desirable to know how much of the literature of other than Chinese origin the author has consulted. It is clear even in this reference-free paper that Professor Lee had access to nothing beyond about 1963. The magnetic stripes off western North America are described in some detail, but what they imply "is not yet clearly known" beyond that basic or ultrabasic rocks are present. Mid-ocean ridge magnetic anomalies are not mentioned. Seismicity only receives the briefest qualitative attention. With this crippling lack of the ingredients that went into plate tectonics (and without the 1961 seafloor spreading hypothesis), Lee's synthesis is bound to be incomplete. He further complains that "reactionary imperialistic blocs" in scrambling for the wealth of the sea floor and other purposes have not published most of their data. Nevertheless his paper has much interest.

He first looks at the various hypotheses of crustal movement. Some geologists—most of the "traditionalist Americans and some orthodox geotectonicists in the Soviet Union" (Belousov, no doubt)—insist that vertical movement is dominant and flatly deny the importance of horizontal movement. This he sees as founded in ideas of continental fixation and thus "metaphysical, and utterly dogmatic. This guiding thought was propagated to our land and has brought considerable loss to our geological cause". Lee then goes on to consider possible aspects of crustal movement—cooling and contraction, tides, expansion, convection currents (not ruled out but a "purely metaphysical hypothesis"), isostatic compensation, continental drift and what is called "China's own road". Most of these are dismissed peremptorily, but continental drift (separate from convection currents) is considered briefly as "not entirely unreasonable". Lee, however, cares little for palaeomagnetism and does not accept field reversals.

China's "own road" is an approach which he, as author of a textbook on the geology of China, finds most appealing: learn what you can from China's "extremely superior conditions of structural development". Extensive field work is necessary on structural theories—as Chairman Mao has it, "Man's knowledge is verified only when he achieves the anticipated results": advice perhaps more suited to debugging computer programs than scientific research. Once Lee discovered certain broad scale structural regularities in China, he was prepared to extend them to the rest of the Earth. These features are:

- A latitudinal system of E-W zones revealed by both topography and aeromagnetics. One is at 40° to 42°N and is seen to extend through Central Asia, Turkey, the Mediterranean, under the Atlantic, intersecting the Appalachians, producing an east-west gravity anomaly in the western United States and running into the Mendocino fracture zone. Other world circling zones at 32°-34°N and 25°N are described and one or two in southern latitudes.

- A meridional system is identified by north-south trends, less obvious in China but strong elsewhere such as in the Urals and East Africa.

- The Neocathaysian system striking NNE-SSW is a feature of importance on the oceanic margins of Asia.

- The Cathaysian (NE-SW) system is again an Asiatic feature.

- Shear structural systems are complex but all reflect areas in which there has been shear in the horizontal plane.

Lee observes that surface features are often not in complete agreement with those at greater depth, "in other words they are detached from each other". Furthermore the upper part of continents is inconsistent with the ocean floor and so he is led to consider the importance of horizontal movement. But of what? Earthquakes in Yunnan and Kweichow are about 10 km deep so it seems that there is a gliding of the upper crust over the lower. North and South America have glided westward from Europe and Africa leaving the Atlantic exposed. In the Pacific it is the ocean floor which is moving westward and the inclined plane of earthquakes beneath Japan and the Kuriles is a manifestation of shearing between the old Pacific and the underside of Asia. Many other instances are given of meridional and latitudinal displacements of the Earth's crust, leading respectively to movement from higher to lower latitudes and to splitting of continents.

What is the driving force? Lee sees forces from the Earth's rotation as the only ones able to produce the two orthogonal types of motion. The resolved tangential component of centrifugal force is seen as the source of movement towards the equator, presumably (though this is not stated) in the form of *Polfluchtkraft*, the force on floating bodies that so occupied German geodynamicists in the 1920s. Westward movement is seen, on the other hand, as the result of variations in the rate of rotation of the Earth.

Very little of all this will move Western geophysicists, utterly committed to plate tectonics, to turn with enthusiasm to the Chinese literature. Lee's view of the world, so heavily dominated by what he saw in the field in China, is reminiscent of other qualitative syntheses that were made in the 1960s, all of which are now forgotten. And yet there is much to learn from this paper. Lee had to develop his ideas in the context of continental tectonics, a field which is still open to much discussion. The paper shows the fierce independence of the Chinese from the influence of the Russian school whose insistence on the dominance of vertical movement in the crust effectively kept the Soviet Union from participating in the excitement of the past ten years. It further shows that although Lee's views were by no means those of the West, there are grounds for discussion with Chinese earth scientists. Students brought up on Lee's interpretations would not find it difficult to understand, if not to accept, plate tectonics.

Markers in heterogeneous nuclear RNA

from our
Molecular Genetics Correspondent

ONE difficulty in following the production of messenger RNA from its putative precursor, heterogeneous nuclear RNA (HnRNA), is the lack of markers which can be traced from the nuclear molecules to those in the cytoplasm. Such markers are needed both in the part of the HnRNA which is conserved and becomes the messenger and also in the part which is degraded. The importance of such sequences is emphasised by the use which they have been in tracing the path of production of ribosomal RNA from its large nucleolar precursor, where methyl groups identify the ribosomal RNA sequences and certain oligonucleotides have been found which may be part of the degraded sequences.

The marker usually taken to identify the mRNA sequence in HnRNA is the 200-long sequence of poly(A) which is added to the 3' end of the molecule before production of mRNA. The experiments which have shown that preventing addition of this poly(A) prevents production of mRNA suggest that it plays some part essential for messenger maturation. But these experiments have relied on drugs which block this process and in such cases it is always possible to argue that some unanticipated side effect of the drug is really responsible for its effect. The experiments now reported by Nakazoto, Edmonds and Kopp (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 200; 1974) therefore achieve two purposes. By identifying short oligonucleotide sequences of adenylic acid within HnRNA, they identify markers in it additional to the poly(U) sequences previously identified by Darnell and his colleagues; by comparing the metabolism of this oligo(A) with that of the 3' terminal poly(A), they confirm that the distinctive metabolism of the terminal poly(A) is indeed due to its position and not to some unexpected property of the drugs used to inhibit its addition.

Two drugs commonly used to follow the metabolism of HnRNA and mRNA are actinomycin and 3'deoxyadenosine (otherwise known as cordycepin). Low doses of actinomycin suppresses synthesis of rRNA but not HnRNA; higher doses inhibit both rRNA and HnRNA synthesis. Cordycepin does not inhibit synthesis of HnRNA, but by preventing the addition of poly(A) to the precursor, also seems to prevent production of mRNA, a strong line of evidence supporting the idea that HnRNA and mRNA have a precursor-product relationship. The oligo(A) recovered from within the HnRNA molecule

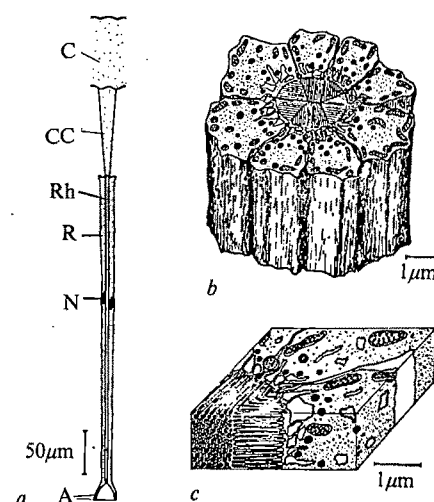
seems to be some 20-40 residues long, compared with the 200 bases of the terminal poly(A). Their metabolism is quite different. High levels of actinomycin which inhibit HnRNA synthesis prevent the synthesis of oligo(A); but have no effect on the addition to previously synthesised HnRNA of the terminal poly(A). Cordycepin has the opposite effect; it has no effect upon the synthesis of the internal oligo(A) but is effective in preventing addition of the terminal poly(A). By comparing these two sequences, these experiments provide strong support for the idea that the terminal poly(A) is indeed added by a mechanism quite distinct from that responsible for synthesis of HnRNA and that its addition is essential for the cleavage from HnRNA of mRNA and its transport to the cytoplasm.

The oligo(A) does not appear in cytoplasmic mRNA and so provides a marker for the sequences of HnRNA which are ultimately degraded in the nucleus. Because these experiments compared internal and terminal poly(A) sequences, they provide information only about the HnRNA molecules which contain terminal poly(A); Nakazoto *et al.* remark that this proportion represents some 15-40% of the total nuclear population. In the largest HnRNA molecules containing external poly(A), Nakazoto *et al.* calculate that there is on average less than one small internal sequence of oligo(A) per molecule. Although present in many of the HnRNA molecules, it is not therefore an inevitable feature. A more detailed examination of its occurrence and location will be necessary.

Miniaturised insect retina

from our Insect Physiology Correspondent

THE more closely the compound eyes of insects are studied, the greater becomes the respect which they inspire. Formerly regarded as primitive and inefficient structures, they are now revealing unsuspected complexities. Some of the latest acquisitions in this field are reviewed and new ones described by Snyder, Menzel and Laughlin (*J. comp. Physiol.*, **87**, 99; 1974) who deal with the functional specialisations of the 'fused rhabdoms' of the insect eye. In most of the photoreceptors of vertebrates and invertebrates the photosensitive pigment resides within the receptor cell in a column of higher refractive index than the surrounding medium. This column of photopigment acts as a dielectric waveguide which confines the light within the photoreceptor, where it is absorbed by the photopigment and transduced to receptor potential.



Structure of a typical cylindrical ommatidium with a fused rhabdom in the honey bee. *a*, General structure showing: C, corneal lens; CC, crystalline cone; R, retinula cells; N, nucleus; Rh, fused rhabdom; A, axons. *b*, Structure as seen with electron microscope: the fused rhabdom at the centre consists of sectors with parallel microvilli—the rhabdomeres of the eight retinula cells. *c*, A segment of the fused rhabdom showing the microvilli of two adjacent retinula cells. The microvilli of each rhabdomere are parallel in a hexagonal lattice.

Thus in vertebrate eyes, with few exceptions, each rod or cone is an independent waveguide, optically isolated from its neighbours. But in most compound eyes of insects several photoreceptors are fused together to form a centrally located common light guide, the rhabdom. In the worker honey bee (see figure), for example, eight photoreceptors (the retinula cells) each contribute a wedge of microvilli (termed a rhabdomere) and these eight wedges are fused together to form the common rhabdom. The parallel microvilli carry the photopigment aligned on the microvillus membrane so that each rhabdomere is sensitive to the plane of polarisation of the incident light. There is increasing evidence that the fused rhabdom is formed by rhabdomeres with different spectral absorptive powers, so that, given the necessary neural connections, spectral discrimination is possible in a single ommatidium. This will make possible a fine-grain colour vision, permitting hue discrimination in a very small field of vision.

But there are more subtle properties of the fused rhabdom which the authors have brought to light. The retinula cells of one cylindrical unit, or ommatidium, can interact both electrically and optically. Electrical coupling between cells occurs, and when this takes place between cells with microvilli aligned in different directions, it will impair the sensitivity to polarised light. On the other hand, if coupling occurs between cells with different photopigments, this

will result in a broadening of the spectral sensitivity curves of each and may be responsible for the double peaked spectral sensitivity curves that have been obtained from single retinula cells.

Optical coupling can take two forms. In some compound eyes, such as those of dragonflies and some Diptera, the rhabdoms are tiered and there is a direct filtering of light through successive photoreceptors with different properties. But even when the rhabdomeres are fused in a single column, since this acts as a single light guide, the absorptive properties of each rhabdomere will influence the light as it passes down the column. Indeed each rhabdomere acts as if it were an absorptive filter in front of all the others: the rhabdomeres function as lateral absorption filters. As a result the shape of the spectral sensitivity curve is more or less independent of the amount of light absorbed: the flattening of curves by self-absorption is prevented by optical coupling. This means that, as a consequence of optical coupling, each retinula cell can have a high absolute sensitivity while preserving its narrow spectral sensitivity curve.

In this way arthropod photoreceptors have solved the problem of collecting the small amount of light available to them and have increased the efficiency of quantum capture without loss of spectral sensitivity. Moreover, unlike electrical coupling, optical coupling within the ommatidium confers these benefits without loss of sensitivity to the plane of polarisation of the incident light. In many forms it looks as though polarisation sensitivity has been a by-product of rhabdom structure without much functional significance. Where a high level of polarisation sensitivity exists, it is found to be the result of a specialised arrangement of rhabdoms to give maximum sensitivity independent of spectral discrimination—as in the ninth retina cell of the honey bee, or the eighth cell in the fly.

In vertebrates the same problem of combining high absolute sensitivity with well defined spectral discrimination has been solved by having a dual system of rods and cones for low intensity and high intensity vision respectively. But it seems that certain deep sea fishes have also developed a system comparable with that of insects in which optical coupling provides a visual system which combines high spatial acuity, absolute sensitivity and colour discrimination in a compact array of photoreceptors behind a dioptric apparatus of extremely low aperture. So far as the insect is concerned, the ommatidium cannot be considered as a loose collection of receptors sharing the same dioptric apparatus; it must be seen as a highly integrated unit with remarkable discriminatory powers.

Carcinogenicity and mutagenicity

from a Correspondent

A WORKSHOP meeting convened by the International Agency for Research on Cancer and the Catholic University of Louvain was held in Brussels on December 10-12 to discuss approaches to assess the significance of experimental chemical carcinogenesis data for man. The meeting was chaired by Dr P. N. Magee (Middlesex Hospital Medical School, London) and the topics included comparative metabolism of chemical carcinogens, chemical carcinogenesis *in vitro* and mutagenicity tests.

Comparative metabolism

A. H. Conney (Hoffman-La Roche, Nutley) discussed the marked species differences in metabolism of foreign chemicals and their importance in determining the outcome of exposure of animals or man to chemical carcinogens. He emphasised the great difficulties and dangers involved in studying the metabolism of carcinogens in humans and the possible value of metabolic studies on non-carcinogenic chemicals. Recent work on metabolism of chemical carcinogens *in vitro* has included the use of biopsy or postmortem samples of human liver and preparations of human placenta, leukocytes or foreskin. E. Arrhenius (University of Stockholm) described how chemical carcinogens are metabolised, usually by microsomal enzyme systems which might activate or inactivate them. N-oxygenation was claimed to activate and C-oxygenation to inactivate aromatic amines, the reactions differing in their dependence on cytochrome P_{450} and in their response to inhibitors such as methylmercury compounds. The active metabolites, usually of electrophilic character, react with components of the cell nucleus and endoplasmic reticulum.

The evidence that N-nitrosamines are activated by enzymes to form alkylating agents whereas N-nitrosamides can form similar products in the body without enzymes was mentioned by R. Montesana (International Agency for Research on Cancer, Lyon). The organotropic action of the nitrosamines may be determined by the distribution of the activating enzymes but metabolism, although necessary, is not a sufficient condition for carcinogenesis. Human liver preparations metabolise dimethylnitrosamine *in vitro* at a comparable rate to rat liver and show a similar pattern of nucleic acid methylation. F. J. Wiebel (National Cancer Institute, Bethesda) described metabolic pathways of polycyclic hydrocarbons which lead to less or more carcinogenically-active products. Epoxides may be the active

metabolites with dihydrodiols, phenols and quinones as less active products. The enzymes concerned are present, and can be induced, in mouse skin and are inhibited by 7,8-benzoflavone which also inhibits skin carcinogenesis. Inhibitory effects of actinomycin D and cycloheximide suggest a role of messenger RNA in the induction of the enzyme. Differences in the activities of aryl hydrocarbon hydroxylase in human leukocytes may provide a basis for predicting susceptibility to these carcinogens in man.

P. L. Grover (Institute of Cancer Research, London) discussed the significance of polycyclic hydrocarbons in tobacco smoke for the induction of human lung cancer, suggesting the possible production of somatic mutations by enzymatically-formed epoxides. Direct evidence, including *in vitro* studies with human lung microsomes, and indirect evidence for formation of epoxides was presented and the importance of their further metabolism by hydrolases and glutathione conjugation was discussed. E. Huberman (Weizmann Institute, Rehovot) described a highly sensitive cell-mediated assay for mutagenesis in mammalian cells in which mutation to 8-azaguanine resistance was scored in V79 Chinese hamster cells, growing on layers of lethally irradiated cells which still retained the capacity to metabolise chemical carcinogens. Increased mutation rates, inhibited by 7,8-benzoflavone, were obtained with benzpyrene and methylcholanthrene. N-methyl-N'-nitro-N-nitrosoguanidine was active without the irradiated cell layer. Mutagenic activity correlated with carcinogenicity. P. T. Iype (Paterson Laboratories, Manchester) described methods for the *in vitro* culture of liver parenchymal cells with characteristic morphological, biochemical and immunological properties including contact inhibition, the presence of desmosomes, glycogen, ligandin, tyrosine aminotransferase and the capacity to produce serum albumin. Transformation was obtained with methylnitrosourea and other chemical carcinogens and confirmed by transplantation.

Testing for carcinogenesis

In vitro models for carcinogenesis testing were discussed by J. A. DiPaolo (National Cancer Institute, Bethesda) who emphasised quantitative experiments using mammalian cells and the production of cancer by transplantation of transformed cells into animals as the end point. Syrian hamster fibroblasts growing on irradiated rat cells were transformed by polycyclic hydrocarbons and the results correlate with carcinogenic potency *in vivo*. Toxicity reactions and transformation were separate and distinct processes. A similar banding pattern was observed in the chromo-

some of transformed cells and tumour cells but these were considered secondary rather than causal. Pretreatment of the fibroblasts by irradiation or with methyl methanesulphonate enhanced the effect of some chemical carcinogens. Some compounds, including nitrosamines, were negative in the *in vitro* system but gave positive results if the embryos from which the fibroblasts were derived were exposed to the carcinogens transplacentally. The replica plating technique of Lederberg has been applied by T. Kuroki (University of Tokyo) to the selection of ultraviolet sensitive clones from mouse cell lines and of mutants induced in BHK-21 cells exposed to N-methyl-N'-nitro-N-nitrosoguanidine.

Testing for mutagenesis

Possible damage to the genetic material of man and animals from exposure to pesticides in the environment was pointed out by R. Fahrig (Zentral-laboratorium für Mutagenitätsprüfung, Freiburg), who described a range of tests for mutagenicity of such environmental chemicals with emphasis on the reasons for negative results. These included insensitivity of the system used, lack of activation of the compound and failure of penetration of the compound to the critical sites in the cells. Little is known about long-term exposure of animals to mutagens. The known liver microsomal activating systems for chemical mutagens were surveyed by N. Loprieno (Institute of Genetics, Pisa) whose group has used forward mutation and mitotic gene conversion and recombination with *Saccharomyces* as the test organism. The mutagenic activity of ethyl methane-sulphonate, a direct alkylating agent, is enhanced by mouse liver microsomes, but this effect was not explained. T. Sugimura (National Cancer Center Research Institute, Tokyo) summarised current work on AF₂, a nitrofurant derivative used for about 7 years as a food additive in Japan. This compound gave positive results for DNA damage in repair tests and was positively mutagenic in *Escherichia coli* although negative in the *S. typhimurium* mutants of Ames. Carcinogenicity testing has so far proved negative and the compound was inactive in the sebaceous gland inhibition test in mouse skin. The great difficulties in extrapolating these results to man were discussed.

The positive correlation between the mutagenicity of selected chemical carcinogens in *Neurospora crassa* and their carcinogenicity in laboratory animals was emphasised by F. J. de Serres (National Institute of Environmental Health Sciences, Bethesda). He described a system of specific locus mutations in *Neurospora* which showed that chemical carcinogens produce

mainly base-pair substitution mutations, by contrast with the conclusions of some other workers that many potent carcinogens induce frame-shift mutations. He concluded that it may eventually be possible to develop a battery of tests that will permit the reliable prediction of carcinogenicity and mutagenicity. Finally, G. Röhrborn (University of Heidelberg) reviewed the mutation theory of cancer, emphasising that most human cancers do not show a definite mode of inheritance, though chromosomal aberrations are seen in human tumours (for example, Bloom's Syndrome) and carcinogens of various kinds interact with DNA. Rats treated with N-nitrosomorpholine or N-butyl nitrosourea showed evidence of chromosomal damage in the bone marrow and work is in progress to develop tests for carcinogenicity and mutagenicity in the same species of animal.

Superconductivity of metastable alloy films

from our Materials Science Correspondent

In the early fifties, Buckel and Hilsch in Göttingen deposited mixtures of metals such as bismuth and tin by evaporation on to substrates cooled by liquid helium, and so made supersaturated alloy films some of which showed superconducting behaviour to unexpectedly high temperatures. The method has been designated vapour quenching. This work has in recent years stimulated a fluctuating follow-up, first at IBM and now at Bell Telephone Laboratories, research which has gone in parallel with the growing amount of research on alloys cooled ultrarapidly from the melt. Both vapour-quenched and melt-quenched alloys are apt to be supersaturated, sometimes grossly so, and are thus in metastable form. Uncovenanted phases, not indicated by the phase diagram, are also apt to be formed.

The group at Bell Telephone have now published a detailed account of their recent researches on the superconductivity of vapour-deposited films (Testardi, Wernick, Royer, Bacon and Storm, *J. appl. Phys.*, **45**, 446; 1974). One novel feature here was that the substrate was not cold, but on the contrary held above room temperature during deposition. Instead of evaporation, the comparatively new method of getter-sputtering was used, in which the vapour species are generated by bombardment of metal targets by argon ions, in an atmosphere cleaned by gettering. The alloys, mostly high-melting compositions with superconducting major constituents, were found to produce metastable phases, as evidenced by X-ray diffraction. Com-

bination such as Hf/Nb, Mo/Nb/Re, Re/W, Rh/Zr were examined.

The superconducting transition temperatures, T_c , are plotted as a function of deposition (that is, substrate) temperature, and the generalisation emerges that T_c peaks for deposition temperatures which are close to phase-transformation temperatures in the equilibrium diagrams. The alloys studied mostly have equilibrium phases of δ , α -Mn or similar structural type near the temperatures which led to the highest values of T_c . The investigators conclude that structural instability enhances the superconducting transition; presumably, the phase which is in process of being formed is one which, in its normal state, is especially favourable for superconduction. But Testardi *et al.* were generally not able to identify with certainty what phases, metastable or otherwise, were in fact present in their films; they did establish that some X-ray lines at least corresponded to abnormal structures. This work shows that getter-sputtering is an effective technique for forming alloy phases with unusual properties, and that this virtue of the technique is by no means restricted to experiments with cold substrates.

At first sight, deposition of these alloys on a heated substrate is entirely distinct from deposition of bismuth or copper alloys on substrates held at 4 K. In fact, the difference is only apparent. Testardi's alloys are so refractory that even at temperatures around 400–600°C, diffusion in the bulk is very sluggish. Where the alloys have phase transitions in this temperature range, the new phases form very slowly when the alloy is cooled from the melt, whereas formation from the vapour permits the newly arriving, momentarily mobile atoms to form themselves readily into the equilibrium phase or a metastable transition phase of related structure. (It seems that the metastable structures form most readily in the immediate vicinity of a temperature at which a phase change would take place according to the phase diagram.) What Testardi and his colleagues have introduced is a form of vapour-quenching at elevated temperatures in which the film is transiently in a labile, adaptable state and is therefore at once frozen into instability.

Two of the alloys investigated, Mo₃Ru₃ and Ru₂W₃, also show exceptional hardness and corrosion resistance (Testardi *et al.*, *Met. Trans.*, **4**, 2195–2198; 1973) which might make such alloy films interesting for use as thin-film resistors, optical mirrors, razor blade coatings and so on. Since these alloys have now also been shown to have useful superconducting characteristics, they may also prove of value for use in superconducting memory devices.

Where does a membrane end?

from a Correspondent

THERE is currently a considerable amount of effort being expended in attempts to define the molecular structure of the surface membranes of cells. Unfortunately, there is no adequate definition of a 'membrane protein' and workers in this field compromise, either explicitly or, more often, implicitly, by adopting operational definitions. Singer and Nicholson (*Science*, **175**, 720; 1972) distinguish between 'integral' and 'peripheral' membrane proteins largely on the basis of their ease of solubilisation from membranes. This is, of necessity, an arbitrary decision: an integral membrane protein is one which remains attached to the fragmented vesicles which usually comprise 'purified membranes'. The problem with this approach is illustrated by the following comparison. A large fraction of the total cellular sialic acid is accessible to trypsin and therefore presumably is at the surface; however, membranes purified by some of the most commonly used procedures (Warren *et al.*, in *Specificity of Cell Surfaces*, edit. by Davis and Warren, 1967) contain only a small fraction of the total sialic acid. Does one conclude that the sialylated molecules are peripheral membrane proteins, or extracellular material, or alternatively, that the membrane preparation is inadequate? Clearly, it boils down to a semantic argument avoided only by operational definitions.

There is also the opposite problem of association, artefactual or otherwise, of non-membrane proteins, for example serum proteins, with cells and membrane preparations. One could propose, as a definition of a true membrane protein, that it be synthesised by the cell itself and possess a hydrophobic region inserted into the lipid bilayer. This definition also has a number of disadvantages; not least that it is, in practice, difficult to determine whether a particular protein is rooted in the lipid bilayer. It would also exclude proteins synthesised elsewhere and inserted into the membrane of a cell. There is clear precedent for such an export-import system in the case of egg yolk proteins which are synthesised in the liver.

Two recent papers illustrate the problem. Peterson, Rask and Lindblom (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 35; 1974) report that HL-A antigens released from 'crude spleen cell membranes' by papain digestion contain the protein β_2 -microglobulin, previously described as a protein present in serum and urine. Peterson *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 1697; 1972) had earlier found that this protein can be

detected immunologically on the surface of human leukocytes. Now they prove that β_2 -microglobulin is associated with several different HL-A antigenic specificities throughout papain treatment, a variety of column chromatographic procedures and immunoprecipitation by HL-A antisera. The β_2 -microglobulin was identified by electrophoretic mobility, antigenicity and tryptic peptide mapping. The HL-A specificities resided in polypeptides of about 33,000 daltons but, in each case studied, were associated with a polypeptide of 11,800 daltons indistinguishable from β_2 -microglobulin. The β_2 -microglobulin did not itself carry HL-A specificities. Does this association represent artefactual binding arising after digestion, or does it pre-exist on the intact cell and what is the origin of the β_2 -microglobulin? No evidence is presented as to whether the β_2 -microglobulin is synthesised by the spleen cells themselves. Previous workers, who have described a polypeptide of similar molecular weight in purified HL-A, found that it was synthesised by the cultured lymphocytes from which it was isolated (Cresswell, Turner and Strominger, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603; 1973).

In an unrelated study Ruoslahti, Vaheri, Kuusela and Linder (*Biochim. biophys. Acta*, **322**, 352; 1973) discovered that an antigenic activity found on the surfaces of cultured chicken embryo fibroblasts is present in chicken serum. In this case the serum protein is of high molecular weight and migrates as an α -globulin. Antisera specific for this antigen were obtained by the unlikely procedure of treating intact fibroblasts with insolubilised papain and injecting the digest (after removal of the enzyme) into rabbits. Surprisingly, a monospecific serum was obtained. Using this serum, Ruoslahti *et al.* were able to show that the antigen is present on the surfaces of the fibroblasts (but not of several other cell types) and is synthesised by them (incorporation of 35 S-methionine). The fibroblasts from which the immunogen was obtained had been cultured in heterologous sera which do not themselves contain the antigen. Antigen was released into the medium of cells cultured in calf serum and its presence in chicken serum would suggest that it is also released from fibroblasts *in vivo*. The concentration of the antigen in chicken serum was estimated at several μ g per ml and is, therefore, much higher than the amounts of histocompatibility antigens and carcinoembryonic antigen which can also be detected in sera.

These two reports both show that one cannot always readily distinguish between membrane and serum proteins, let alone between 'true membrane proteins' and 'extracellular proteins'. In the

case of the fibroblast surface antigen the obvious interpretation is that the cells synthesise the antigen which, after a period at the cell surface, falls off into the serum. Whether the situation is the same or the reciprocal in the case of β_2 -microglobulin, it is clear that no firm line can be drawn which defines certain molecules as membrane proteins and others as non-cellular. The membrane rather than having an end, tails off into the medium. This is not a doctrine of despair for those hoping to define the molecular composition of cell membranes. Rather, it suggests the need for a more precise description of the molecular arrangement in temporal as well as topological and topographical terms.

Earth spin theory queried by doubting Thomas

by our Cosmology Correspondent

THE Ordinary Meeting of the Royal Astronomical Society held on March 8 was notably lively and lighthearted. The tone was set early in the proceedings, when the secretary, reading out the list of presents received encountered the item "De Witt and De Witt, 'Black Holes', presented by Mr Grey"; this provoked an appropriate audience response.

Even after the preliminaries, however, things did not immediately settle down into the technicalities of specialist papers; the first speaker, D. D. Thomas (Royal Greenwich Observatory), described "recent variations in the rotation period of the Earth", a topic which clearly aroused great interest among the majority of the fellows present, whatever their own specialist interests. The reason for presenting this item at this particular meeting was, Thomas said, the excessive press interest in the change in the length of the day (LOD) which occurred in December 1973.

As Thomas explained, this jump in the LOD was far from being an unusual event. A similar effect occurred in the preceding winter, and changes of the order of a millisecond in the LOD are not uncommon, although it is true that they occur more frequently in summer than in winter. And indeed the December 1973 event may not even have been very sudden, since the data show only that it occurred within a period of about 3 weeks. So why did it arouse such interest? Perhaps, Thomas suggested, the press were unusually sensitive to astronomical stories at that time, because of Comet Kohoutek.

Thomas then went on to discuss the variations in the LOD in more general terms. It is now generally accepted that seasonal changes in the rate at which

the Earth spins are caused by seasonal changes in the general circulation of the atmosphere, and he argued that meteorological effects could well be responsible for sudden changes in the LOD as well. He cast doubt on the reliability of evidence that earthquakes or sunspots might be causes of changes in the LOD. This is not to say that the overall trend in the acceleration of the Earth could not be influenced by solar activity, but "it is dangerous to argue a causal relationship between specific solar events and changes in the LOD".

Around the time of the recent Earth spin event, there were, it seems, changes in the pattern of westerly winds at the 500 mbar level, but Thomas declined to be drawn onto dangerous speculative ground, closing his talk with an emphatic statement that he would not attempt to say what causes the meteorological variations. As readers of the News and Views section will be aware, however, there is now compelling evidence that solar activity affects the very meteorological processes which seem to affect the LOD (see *Nature*, 246, 384; 1973).

Earth plates as membranes

from our Geomagnetism Correspondent

In recent years several workers have sought to explain the origin of major tectonic activity away from plate boundaries in terms of hot spots, with or without associated mantle plumes. The best known example of the use of such an interpretation is probably the Hawaiian-Emperor island chain which is seen as the surface trace resulting from the motion of the Pacific plate over a stationary or near stationary magma source; but continental graben and rift valleys, such as the Rhine graben, have also been associated with hot spots. On the other hand, there are problems with this viewpoint and there are alternative, if no less contentious, explanations for both continental and oceanic mid-plate tectonic structures. The Rhine graben, for example, has an average width of 36 km and an estimated extension across the valley of 4.8 km; but it is not entirely clear how such features could result from hot spot or plume activity. And as far as the formation of the Hawaiian islands is concerned, the hypothesis that the chain derives from a propagating tensional fracture in the lithosphere which causes volcanic activity as it extends was put forward as early as 1942 and still receives much support today.

The chief difficulty with both the older and newer interpretations of the Hawaiian chain lies in substantiating them, although the former was recently

given a significant boost by Turcotte and Oxburgh (*Nature*, 244, 337; 1973) who offered two possible origins for the required tensional stresses. The first of these involved thermal effects. Lithosphere created at an oceanic ridge by the cooling of mantle material cools further and increases in thickness as it moves away from the ridge. Such an elastic plate cooled non-uniformly will be subject to thermal stresses in the form of tensions which are parallel to the ridge, and thus aligned with the corresponding magnetic anomalies, but normal to fracture zones. Turcotte and Oxburgh were able not only to calculate the thermal stresses and show them to be sufficient to fracture the lithosphere but also to predict the direction of the tensional thermal stresses near Hawaii and show it to be in close agreement with a similar prediction using the theory for the plastic yielding and fracture of elastic solids.

The second type of tensional stress, the theory of which has now been developed at greater length by Turcotte (*Geophys. J.*, 36, 33; 1974), is the membrane stress arising from non-spherical plate tectonics. The basic point here is that if the Earth were a perfect sphere plates would be able to move about without deformation but, because the Earth is in fact an oblate spheroid with an ellipticity of 0.00335, the lithosphere must deform when its latitude changes. A small plate at the equator, for example, will have two principal radii of curvature (6,378 km and 6,335 km), both of which will increase if the plate moves towards higher latitudes and both of which reach 6,400 km for a plate at either of the poles. Conversely, a small plate moving towards the equator will have its principal radii of curvature decreased. Normally such changes in the radii of curvature would produce bending stresses, but because the surface plates in this case are thin compared with the Earth's radius they will behave as thin shells in which the bending stresses may be neglected in comparison with the membrane stresses arising from stretching the plates to different radii of curvature.

In principle, therefore, it should be possible to determine the membrane stresses associated with the deformation of the Earth's moving plates using the already developed theory of thin shells—and this is what Turcotte has tried to do. For an unstressed thin shell whose radii of curvature are increased, the result will be a tension at the edge and a compression in the interior; for a decrease in the radii of curvature the situation will be reversed. But, first, do such membrane stresses actually exist in real plates? Plates move with velocities in the range 1-10 cm yr⁻¹, thus requiring about 10⁸ yr to undergo a significant change in latitude. It is usual

to regard the lithosphere as an elastic medium on a short time scale; but on the geological time scale will plastic yielding occur, relieving the membrane stresses? As Turcotte points out, at "modest" depths plastic yielding is to be expected because of the higher temperatures involved. But he also notes that the relief of membrane stresses requires plastic flow throughout the whole thickness of a plate and that there is abundant evidence (for example, from major fault systems) for the absence of long term plastic flow in near surface rocks.

Having disposed of this point (which is not the Aunt Sally it might at first seem), Turcotte goes on to develop the theory of membrane tectonics, generally along the lines mapped out by Novozhilov (*The theory of Thin Shells*, Noordhoff, 1959). For the real Earth the determination of the membrane stresses is, of course, an insolubly complex problem; surface plates have irregular shapes and each element of each large plate has its own radii of curvature which change in accordance with the particular variation in latitude. Turcotte thus adopts a highly simplified model in which the initially unstressed plate is represented by a circular segment of a spherical shell and in which the deformed plate is a similar spherical dome but with a slightly larger or smaller radius of curvature. In this scheme, deformation is produced by a radial surface force on the shell.

What emerges from this analysis is that a change in the latitude of the modelled lithosphere can result in membrane stresses of up to several kilobars. The stress required to produce a fracture in the real lithosphere is not precisely known, although stresses on active fault zones are known to be of the order of 0.1 kbar and laboratory work has shown the strength of mantle rocks to be of the order of 10 kbar. It may thus be fairly safely presumed that membrane stresses of several kilobars would indeed be sufficient to fracture the lithosphere, although thermal stresses and the stresses driving the plates may also play a part. Turcotte further shows that membrane stresses of about 2 kbar should lead to gravity anomalies of the order of 10 mgal, which is the magnitude of the anomalies actually observed.

Whether or not the importance of membrane stress is substantiated, Turcotte's analysis can only strengthen the hand of workers such as Jackson and Wright (*Petrology*, 11, 405; 1970) who see the Hawaiian chain in terms of a propagating fracture rather than a hot spot. Moreover, membrane stresses seem to be the more capable of explaining the finite extension of rift valleys—as the extension needed to relieve the stresses.

West Atlantic abyssal circulation during the past 120,000 years

Detmar Schnitker

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Three faunal assemblages of benthic foraminifera are associated with the three abyssal water masses of the West Atlantic. Faunal distribution 15,000 yr ago indicates cessation of Arctic bottom water flow and a modified supply of Antarctic Water during the last full glacial period, resulting in an apparent warming of the bottom water. Faunal distribution and thus the deep sea circulation were similar to present conditions during the last interglacial 120,000 yr ago.

STUDIES of the remains of planktonic microorganisms from deep sea sediments reveal the climatic and oceanographic fluctuations that occurred in the surface waters of the world's oceans during the late Tertiary and particularly the Quaternary¹⁻⁶. Early studies of the abyssal benthonic foraminifera pointed to an apparent homogeneity of the deep-sea faunas⁷⁻⁹. Probably the past experience of micropaleontologists working within small areas and with very variable shallow water faunas made them insensitive to, or suspicious of, the small but real differentiations that can be found in deep-sea material.

Living (preserved) and total (empty shells) benthonic foraminiferal faunas were studied from West Atlantic sediment samples. Among the approximately 150 species of calcareous foraminifera of this area, three consistently recurring assemblages can be recognised whose areal distribution is shown in Fig. 1. Each of these assemblages is named after its most conspicuous member species and is closely associated with one of the three abyssal water masses that are present in the West Atlantic Ocean. The first, or *Epistominella exigua* fauna, occurs in its pure form in samples north of 45°N latitude only and is contained within the contours of the 1.9° C potential temperature of the Arctic bottom water, as shown by Worthington and Wright¹⁰. The second faunal assemblage, dominated by *Osangularia umbonifera*, is best defined within the 1.5° C potential temperature contour of the Antarctic bottom water, south of 30°N latitude. The transition from the *E. exigua* fauna to the *O. umbonifera* fauna is at approximately 35°N latitude, the area where the Arctic and Antarctic bottom water meet and lose their identity¹⁰. That indeed the water characteristics and not bathymetry control the faunal distribution is shown by the *Epistominella exigua* fauna which occurs at shallower depths in the northern portion of its range than further south, thus paralleling the descent of the Arctic bottom water from 500 m at the Denmark Straits to nearly 5,000 m north of Bermuda. Samples located on the continental rise and slope and on the mid-Atlantic ridge contain faunas in which several species of *Hoeglundina*, *Uvigerina* and *Gyroidina* are abundant. With a few exceptions these samples are contained between the 2° C and 4° C potential temperature contours. These faunal assemblages are associated with the lower North Atlantic deep water.

Disagreement between the postulated association of faunal assemblages and water masses appear to exist for four samples

collected within the Gibbs fracture zone, at the southern end of the Reykjanes Ridge. These samples contain typical *Epistominella exigua* assemblages, thus inferring the presence of Arctic bottom water at these sites. According to Worthington and Wright¹⁰ this area should be occupied by North Atlantic deep water. An easterly flow of bottom water through the fracture zone has recently been suggested^{11,12} and is supported here by faunal evidence. In a few areas, such as on portions of the Hatteras and Nares abyssal plains, the distribution of bottom faunas has been inferred only: carbonate solution at these great depths has modified the composition of the faunal assemblages and, in a few samples, nearly eliminated them altogether.

The information gained from the distribution of the present faunas can be applied to those of the past. Figure 2 shows again the distribution of three faunal assemblages for the latter part of the last glacial period. The core samples were taken from the upper part of the y-zone of Ericson *et al.*⁵, or the 17,000 yr BP level of the Climate/Long Range Investi-

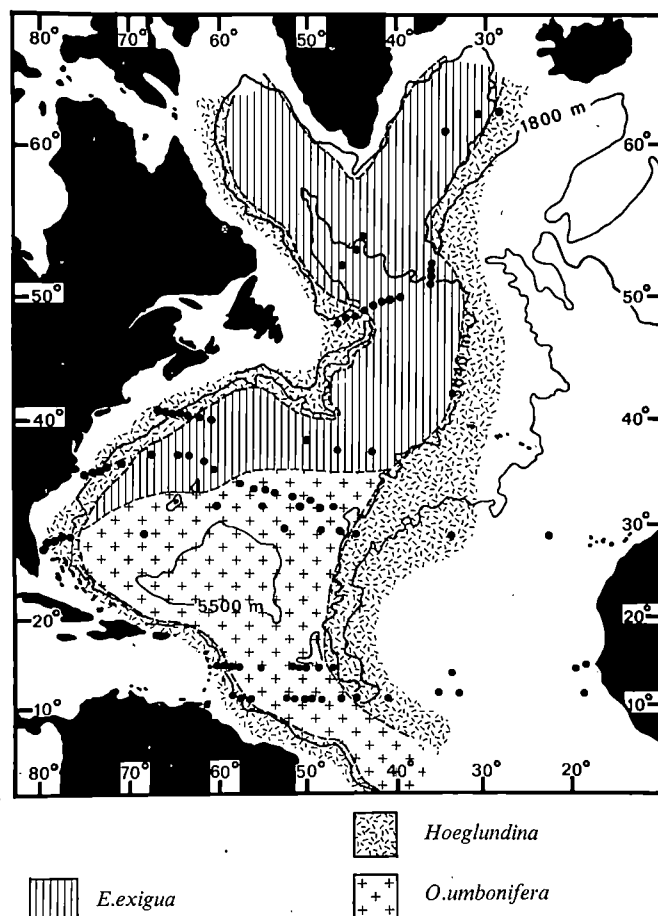


FIG. 1 Distribution of foraminiferal assemblages in the present western Atlantic Ocean. ●, Surface sample.

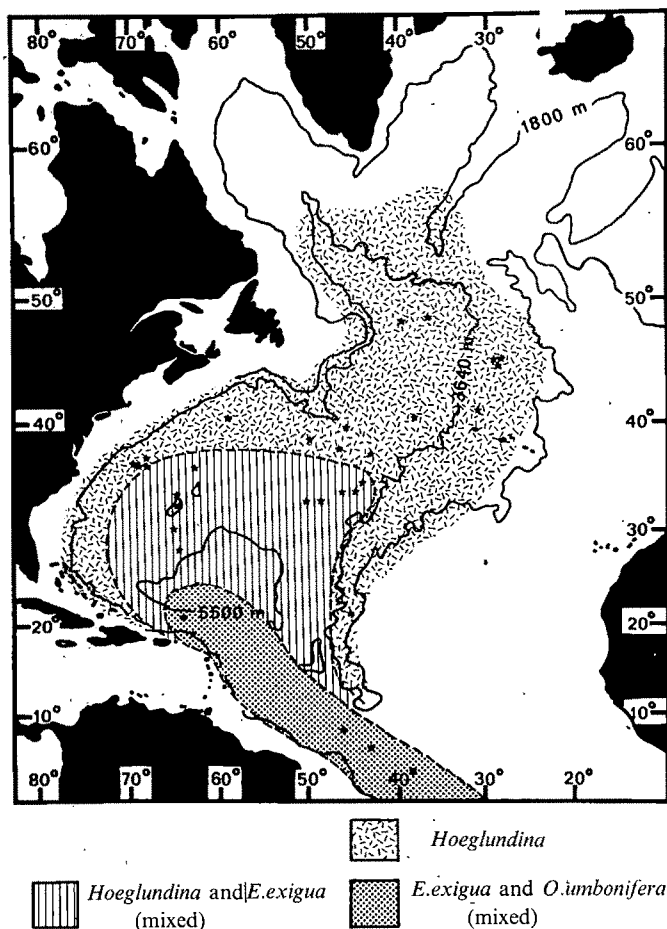


FIG. 2 Distribution of foraminiferal assemblages under glacial conditions, 17,000 yr ago. ★, Core location.

gation Mapping and Predictions (CLIMAP) programme. Only one of the faunal assemblages of the present time existed during late glacial times, the *Hoeglundina* fauna, which at this time has invaded nearly all of the western Atlantic basin north of 40°N latitude. Towards the south, more and more elements of the *Epistominella exigua* fauna are represented so that south of 40°N latitude the bottom fauna gains a new identity as a *Hoeglundina-Epistominella exigua* mixed assemblage. Further south the faunal characteristics change again through a progressive increase of elements of the *Osangularia umbonifera* fauna and a concomitant decrease of the *Hoeglundina* fauna, so that south from about 22°N latitude the bottom fauna can be termed the *Epistominella exigua-Osangularia umbonifera* mixed assemblage.

Such a change in faunal composition and distribution was probably brought about by a change in the deep water circulation pattern and/or a change in the character of the water masses. Apparently, Arctic bottom water was not present and Antarctic bottom water, if it continued to enter the region, must have had characteristics different from those of modern Antarctic bottom water. A water mass with characteristics similar to those of modern Atlantic deep water occupied the entire northern portion of the basin, whereas a water mass which was probably produced by mixing of the northern and southern waters occupied the central portion of the basin.

Figure 3 shows the faunal distribution pattern for a time approximately 120,000 yr ago, towards the end of the last interglacial stage. The faunal assemblages and their distributions are nearly identical to those of today.

Such drastic changes of the deep thermohaline circulation between glacial and interglacial stages within the Atlantic Ocean have been proposed by Weyl¹³: reduced evaporation

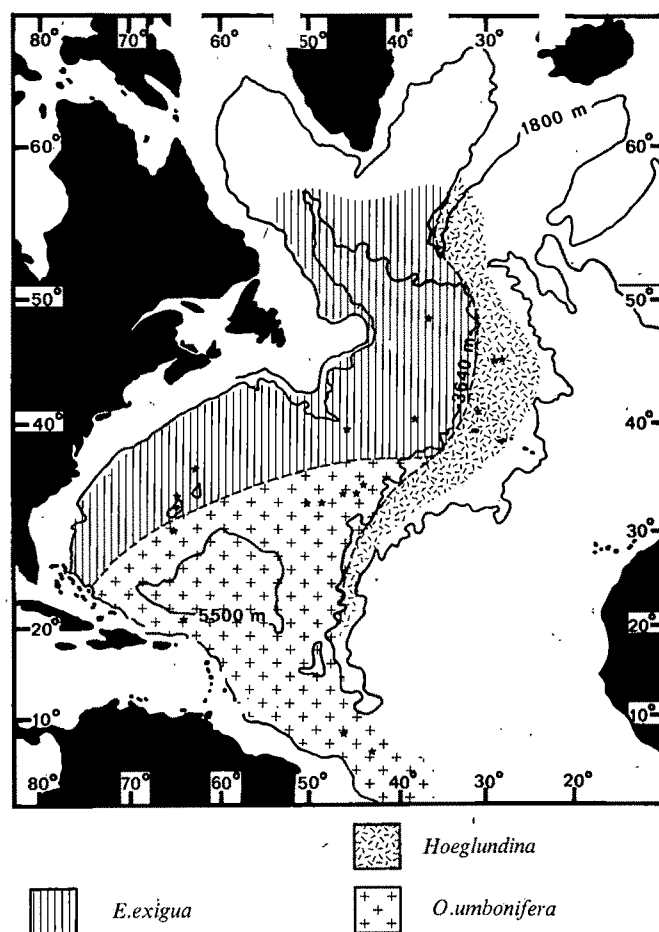


FIG. 3 Distribution of foraminiferal assemblages under interglacial conditions, 120,000 yr ago. ★, Core location.

in low latitudes led to the inflow of lower salinity water into the Norwegian Sea which then acquired a stable density stratification and a cover of sea-ice. Formation of Arctic bottom water ceased. A similar density stratification in the Antarctic would also have prevented the formation of Antarctic bottom water. A subpolar gyre was to form between the sea-ice front, south of Iceland and the subtropical

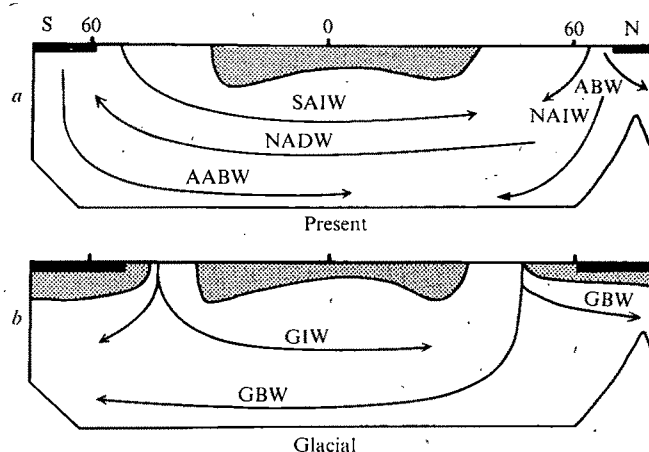


FIG. 4 Schematic N-S diagram of thermohaline circulation in West Atlantic Ocean under, a, interglacial and, b, glacial conditions. Modified from Weyl¹⁰. [E], Low density surface water; ■, sea ice. SAIW, South Atlantic intermediate water; NADW, North Atlantic deep water; AABW, Antarctic bottom water; NAIW, North Atlantic intermediate water; ABW, Arctic bottom water; GIW, glacial intermediate water; GBW, glacial bottom water.

convergence, then at about 40° to 45°N latitude. Glacial bottom water which formed in this subpolar gyre, with temperatures similar to those of the present Atlantic deep water, was to be the only bottom water of the entire Atlantic Ocean. Figure 4 shows the generalised scheme of the Atlantic deep water circulation, modified from Weyl¹³, for glacial and interglacial conditions.

In the previous discussion specific qualities, such as temperature, salinity or dissolved oxygen content, have been purposely avoided and the general term 'water mass' used instead. Experience suggests that temperature may well be the most significant factor controlling the faunal composition and distribution. The present-day transition from the *Epistominella exigua* fauna to the *Osangularia umbonifera* fauna, however, takes place where both the Arctic and the Antarctic bottom water masses have reached a potential temperature of 1.9° C. The temperature in the transition area being equal for the two water masses, other factors, such as salinity or dissolved oxygen must be responsible for the faunal differentiation. The analytical techniques developed by Imbrie and Kipp⁶ may permit a quantitative assessment of the various environmental factors.

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(Duke University) and the RV *Gulliss* (University of Miami). Core samples were made available by the Bedford Institute of Oceanography and the Lamont-Doherty Geological Observatory of Columbia University.

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Nematocyte migration in *Hydra* influenced by tissue polarity

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In Hydra, migration of the desmoneme nematocytes takes place only in the direction of tissue polarity as defined by which end will regenerate a head.

THERE are many examples of cells that migrate as individuals through tissue or water to specific sites. Some of the influences involved in the apparently directed migration of these cells have been elucidated. Chemotaxis has been demonstrated in the aggregation of slime mould cells¹, in the movement of sperm toward the gonangium in the hydroid, *Campanularia*², and in the migration of leukocytes and lymphocytes to areas of infection^{3,4}. The orientation of the substratum upon which the cells migrate is known to orient the movements of cells *in vitro*⁵, and may well play a similar role in the organism. In other cases the movement may not be directed. Instead, cells migrate randomly until they reach the appropriate tissue where specific cell-cell contacts are made preventing any further movement. This mechanism has been postulated for the migration of primary mesenchyme cells in sea urchin embryogenesis⁶, and for the reconstitution of tissue layers from aggregates of cells^{7,8}. Here we describe an unusual type of influence which affects the migration of one type of nematocyte in *Hydra*, namely the polarity of the tissue. The mechanism underlying this influence may eventually prove to be one of those described above.

In *Hydra attenuata* four types of nematocytes (stinging cells) arise by differentiation from interstitial cells in the ectodermal layer along most of the body column^{9,10}. Each type is easily identifiable by the distinctive shape of its nematocyst. More than 80% of the mature nematocytes (H. R. B., and K. Flick, unpublished) migrate through the ectodermal layer¹¹ into the tentacles where they are mounted in pockets in the epithelial cells of the ectoderm. Desmoneme and atrichous isorhiza nematocytes are found only in the tentacles, whereas stenotele and holotrichous isorhiza nematocytes are mounted in the ectoderm throughout the body as well as in the tentacles.

Grafting and nematocyst migration

The large % of nematocytes in the tentacles suggests that when the nematocytes become mature they preferentially migrate in an apical direction. Vögeli¹² has shown that, indeed, small non-epithelial cells in the ectoderm do migrate preferentially in an apical direction. We have obtained more detailed evidence that supports this view. We grafted upper halves of *Hydra* (H123 in Wolpert's scheme¹³) labelled with ³H-thymidine to unlabelled lower halves (4B56F), and the reciprocal of labelled lower halves grafted to unlabelled upper halves. By periodically thereafter examining the unlabelled halves for labelled nematocytes, we found that nematocyte migration is strongly biased in an apical direction (R. L. H., and H. R. B., unpub-

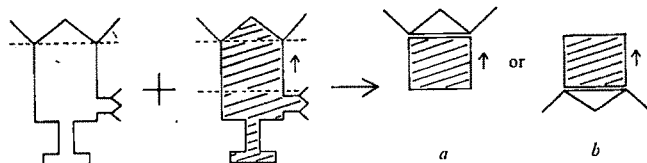


FIG. 1 Grafting procedure for analysing the migration of labelled nematocytes with or against tissue polarity. Dashed lines indicate sites of cutting. The pieces of tissue were removed and grafted together with the head at either *a*, the apical end of the gastric region or *b*, the basal end of the gastric region. Animals (cross-hatched) were labelled by injecting ^3H -proline into the gastric cavity one day before grafting.

lished). In fact, basal migration was found only if a bud was present, and then the nematocytes migrated into the bud. Migration into the peduncle is almost non-existent. These experiments indicate that the migration pattern of nematocytes is not simply one of random movement coupled with entrapment in the battery cells of the tentacles, but actually is one of preferential apical migration.

Once having reached the tentacles, nematocytes remain there and do not diffuse or migrate back into the body column. Campbell¹² has shown that by grafting a head containing nematocytes labelled with ^3H -thymidine to an unlabelled body that there is no measurable migration of labelled nematocytes into the body region. Further, large numbers of septate desmosomes between nematocytes in pockets of epithelial cells and the epithelial cells have been seen^{12,14} suggesting that once a nematocyte is mounted in a pocket it is permanently immobilised. Thereafter, the nematocyte remains on the tentacle until discharged or sloughed off with its host battery cell at the end of the tentacle. As the tentacles are in continuous growth¹¹ and the entire tentacle is renewed in about 6 d (H. R. B., and K. Flick, unpublished), the turnover of nematocytes is relatively rapid.

Chemotaxis or tissue polarity?

The apical directionality of migration may simply be due to a chemotactic attraction towards the head, or it may be due to some property of the tissue in the body column. The two possibilities can be distinguished by grafting a head to the basal end of a piece of the body column. In this case, if nematocytes are to migrate into the head, they must migrate in a basal, not the usual apical, direction. If nematocytes are guided by chemotaxis to the head, the position of the head at the apical or basal end should not affect rates of accumulation of nematocytes in the tentacles. If, however, some relatively stable property of the tissue influences the nematocytes to move selectively in an apical direction, one would expect to find much less accumulation of nematocytes in a basal head compared to an apical head. The following experiment was carried out to examine this.

Unlabelled heads of *H. attenuata* (hypostome and tentacles) were grafted to either the apical or the basal end of the gastric region of animals labelled with ^3H -proline (Fig. 1). In basal grafts, the host head was removed 3–5 h after grafting which inhibited regeneration of a head at the apical end during the course of the experiment. Wilby and Webster¹⁵ found similar results for basal grafts of *H. littoralis* while Wolpert *et al.*¹⁶, also using *H. attenuata*, left the host head on for 8 h to prevent regeneration. The nematocyst capsules were heavily labelled as was expected, since proline and hydroxyproline occur in large quantities in these structures¹⁷.

The accumulation rates of labelled desmonemes and stenoteles were measured as follows: periodically the heads

of five to eight grafts were removed and dissolved in 0.1 N NaOH plus 2.5% sodium dodecyl sulphate. The nematocysts, the only structures which survive this treatment, were analysed autoradiographically to determine the % of desmoneme and stenotele nematocysts that were labelled. The analysis was limited to these two types because they occur in greater numbers than do the two types of isorhizas and their distributions in the animal differ: desmonemes are mounted only in the tentacles whereas the stenoteles are mounted along the body column as well as in the tentacles. It should be noted that the time required for a mature nematocyte to migrate from the body column into the tentacles is short (less than 6 h), (R. L. H., and H. R. B., unpublished), and therefore, is not a factor in this analysis. The results (Fig. 2) indicate that the stenoteles accumulate at the same rate in the tentacles of a head grafted to the basal end of the gastric region as in one grafted to the apical end. In contrast, desmonemes accumulate in the tentacles of an apical head at a greater rate than in the tentacles of a basal head during the first three days. The difference between the rates of accumulation in apical and basal heads is significant (*t*-test, $P < 0.01$) on days 2 and 3, but not on any other day. Desmoneme migration, therefore, appears to be polarised. The fact that desmonemes accumulate at all in basally grafted heads, and the increasing migration rate into these heads after 3 d, might be interpreted, as the result of a gradual reversal of the direction of desmoneme migration under the influence of the basally grafted head.

Regeneration polarity

In this connection it is interesting to consider the effect of basally grafted heads on regeneration polarity in *Hydra*. The tissue of the body column is said to be polarised, as a piece of the column when removed will under normal circumstances always regenerate a head at the apical end and a foot at the basal end. Using *H. littoralis*, Wilby and Webster¹⁸ grafted heads to the basal ends of isolated gastric regions, removed the heads after a variable period, and examined the regeneration characteristics of the original gastric regions. They found that the pieces left in the graft combination for 1–2 d subsequently regenerated with the original polarity, that is, a head at the apical end and a foot at the basal end. After 4–5 d in the graft, the head regenerated at the basal end and a foot at the apical end. Thus, the basally grafted head reversed the regeneration polarity of the tissue. We repeated this experiment with *H. attenuata* (Table 1) and obtained similar results: regeneration with the original polarity after 1–2 d in the graft combination; intermediate forms such as bipolar animals

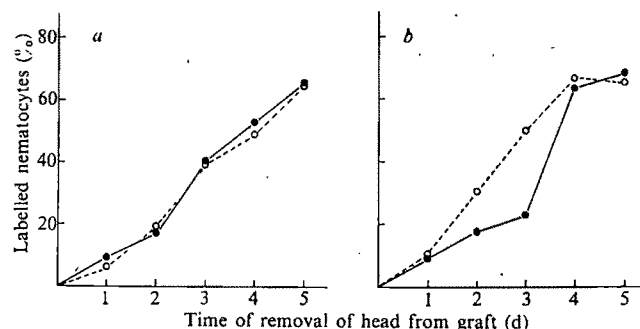


FIG. 2 Rate of accumulation of (a) stenoteles and (b) desmonemes in heads grafted to either the apical end (O) or the basal end (●) of a gastric region. The experiment was carried out twice. Each point represents the average value of 10–15 heads analysed individually. The difference between the rates of accumulation of desmonemes in apical and basal heads is significant (*t* test, $P < 0.01$) on days 2 and 3, but not on any other day.

TABLE 1 The effect of basal head grafts on host polarity

Time of proximal head removal (h)	No. of grafts	Original polarity	Type of regeneration and Apical head		Reversed polarity
			proximal tentacle	Bipolar	
24	10	7	1	2	0
48	10	5	1	3	1
72	10	2	2	5	1
96	7	2	0	1	4
120	7	0	0	1	6

For basal head grafts, the host head was removed 3–5 h after grafting a head to the basal end of the gastric region. As had been demonstrated earlier (R.L.H., and H.R.B., unpublished), removing the host head at this time instead of at the time of the basal head graft prevented normal head regeneration at the apical end. During the course of the experiments, which were carried out at $20 \pm 1^\circ\text{C}$, the animals were not fed, they did not bud, and no head regeneration at the apical end occurred while a head was attached to the basal end of the gastric region.

The four regenerating forms are defined as: original polarity, head regenerating at apical end; apical head and basal tentacle, head regenerates at the apical end and a single tentacle forms at the basal end; bipolar, heads regenerate at both ends; reversed polarity, a head regenerates at the basal end. The table represents the pooled data of two experiments.

(a head at both ends) after 2–3 d; and after 4–5 d in the graft, reversal of polarity. The intermediate forms were to be expected since the reversal of polarity is a gradual process: tissue near the basally grafted head is reversed sooner than the tissue near the original apical end or the gastric region as a whole (R. L. H., and H. R. B., unpublished). The kinetics of polarity reversal seem to parallel the kinetics of desmoneme migration into a basal head: slow at first, and rapidly approaching 100% after 4 d. Thus, the results are consistent with the idea that the direction of desmoneme migration is correlated with the regeneration polarity of the tissue.

A second experiment similar to the first provided more evidence supporting this hypothesis. In addition to grafting a head to the basal end, a peduncle was grafted to the apical end (Fig. 3). Wilby and Webster¹⁸ showed that this combination would reverse the regeneration polarity of the gastric region in 1–2 d instead of the 4–5 d necessary if only a head were grafted to the basal end. When we repeated this experiment with *H. attenuata* (Table 2), we also found rapid polarity changes: after 24 h, only one of 11 gastric regions regenerated according to its original polarity; polarity reversal was observed in more than half of the cases. The rates of nematocyte accumulation into apical and basal heads were measured as before. In this case (Fig. 4) there is no indication that the desmonemes migrate accord-

TABLE 2 The effect of basal head and apical peduncle grafts on host polarity

Time of head and peduncle removal (h)	No. of grafts	Original polarity	Type of regeneration			Reversed polarity
			Bipolar	Bipeduncle		
24	11	1	2	2		6
48	13	1	1	1		10
72	13	1	0	0		12
96	15	0	0	0		15

Both head and peduncle were grafted to the gastric region at the same time in both combinations. During the course of the experiments, which were carried out at $20 \pm 1^\circ\text{C}$, the animals were not fed and they did not bud.

The regenerating forms are the same as described in Table 1. Bipeduncle represents a peduncle which regenerates at both ends and in which no head is formed at all. The data is the sum of two experiments.

ing to the original regeneration polarity. If anything, the rate of accumulation of desmonemes is slightly greater in the basal than in the apical head, but the difference is not significant (t test, $P < 0.05$, except at 2 d). As before, the accumulation rate of stenoteles is the same for a head at either the basal or apical end of the piece of tissue.

Regeneration gradients

The correlation of regeneration polarity and direction of desmoneme migration which we have observed in two experiments suggests that desmonemes may be guided by the 'gradients' which are thought to control regeneration. Little is known about the nature of these gradients although several hypotheses have been put forward^{19–23}. In Wolpert's terms one could say that the desmonemes 'measure' the positional value of the cells they are moving over and migrate only in the direction of increasing positional value (with respect to head formation). During the reversal of polarity because of the basal head graft, the gradient of positional value is inverted, and the desmonemes continue to migrate 'up' the gradient, which is now towards the basal end of the gastric region. In this regard, our results also provide evidence that the changes which occur during the reversal of regeneration polarity of a gastric region occur throughout the piece, rather than being confined to the terminal cells which actually produce the new head and foot.

The mechanism underlying the active migration of desmonemes into the tentacles, or the 'measurement' of positional value, could be based on contact guidance, a restricted form of random movement (excluding basal migration), or some form of chemotaxis. The last is initially attractive because there is some evidence that diffusible substances play a role in the gradients of *Hydra*^{19,22} as well as in other systems such as the insect epidermis^{24,25} but it is doubtful that the desmonemes respond directly to these substances.

In *Hydra*, inhibition of head formation behaves as a gradient of substance diffusing from the head region. The gradient is very labile, vanishing within several hours after removal of the head²⁶. Also, grafting a head to the basal end of a piece of the gastric region establishes a new inhibition gradient, this time with the high point of the basal end, within 3–8 h (see above and refs 15 and 16). If the desmonemes were directly influenced by the gradient of inhibition, or gradient of positional signal¹⁹, one would expect them to migrate into basal tentacles in large numbers after 8 h, and not after 3 d as was observed. Because the properties of the inhibition gradient are not consistent with the observed desmonemes migration behaviour, the desmonemes probably do not 'read' this gradient.

Another possibility is that the desmonemes may respond to a substance diffusing from a graded distribution of sources along the length of the body column, whose density is highest at the apical end²². However, the rapid change (24 h) in tissue polarity in the second graft combination that we and Wilby and Webster¹⁸ observed would require an equally rapid reversal in the graded distribution of sources, or cell

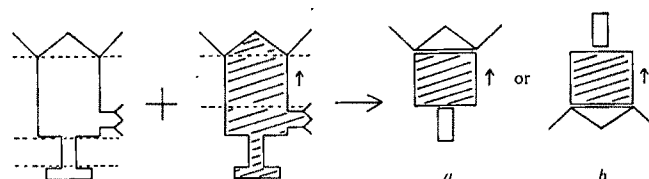


Fig. 3 Grafting procedure for analysing the effect of the peduncle on accumulation rates of nematocytes in basal or apical heads. Dashed lines indicate sites of cutting. The head and peduncle were grafted to the gastric region either *a*, in the original orientation or *b*, in the inverted orientation. Animals (cross-hatched) were labelled by injecting ^3H -proline into the gastric cavity.

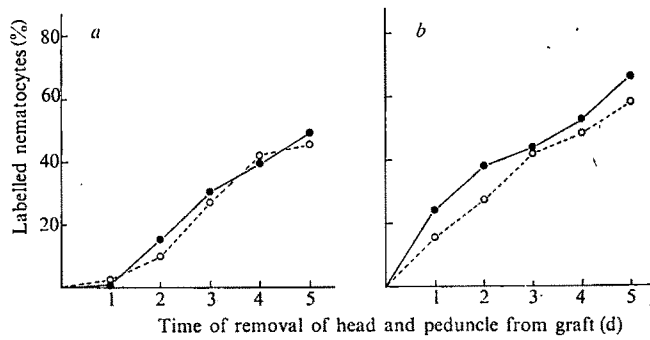


FIG. 4 Rate of accumulation of stenoteles (a) and desmonemes (b) in heads grafted to either the apical end (O) or the basal end (●) of a gastric region in the presence of a peduncle grafted to the opposite end. The experiment was carried out twice and each point represents the average value of 10–15 heads analysed individually. For the desmonemes, the differences between control and experimental points are not significant at the $P = 0.05$ level, but the second day point is significant at the $P = 0.02$ level.

type, which seems unlikely. This evidence would also appear to render untenable any theory in which tissue polarity is due exclusively to the graded distribution of a particular cell type.

Stenotele migration

In contrast to desmoneme migration, stenotele migration is not affected by the polarity of the tissue. Stenoteles will migrate into basally-grafted heads as rapidly as they will into apical heads. This is not simply the result of random movement coupled with entrapment in the tentacles for, as mentioned earlier, in normal animals stenoteles migrate almost exclusively in an apical direction. Only if a bud is present will stenoteles migrate basally (R. L. H., and H. R. B., unpublished), as stenoteles always migrate toward a head region, some form of chemotactic attraction to the head is probably involved.

Thus, desmoneme and stenotele migration are, at least in part, affected by different influences. Though other hypotheses can be constructed to explain the rates of ac-

cumulation of desmonemes in apical and basal heads, these hypotheses must take into account the rather different rates of accumulation of stenoteles and desmonemes in the basal heads in the first experiment. This renders unlikely those hypotheses based on a systematic difference in the experimental design, for such a difference would be expected to affect both nematocyte types in the same way.

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Super-relativistic phase velocities of radio source components

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A sudden injection of relativistic electrons at the centre of an extended dipolar magnetic field could lead to an apparent faster-than-light expansion of two synchrotron emitting regions. Subsequent injections of electrons would produce components which move outwards along the same axis.

INTERFEROMETRIC radio observations with transcontinental baselines reveal rapid fluctuations in the fine structure of some extragalactic radio sources,^{1,2} and in at least three cases these fluctuations imply expansion of small radio components at

velocities apparently greater than that of light, if these sources are at the distances indicated by the cosmological interpretation of their redshifts. In the case of the QSOs 3C279 and 3C273, the apparent separation velocities of double components are 6c and 4c respectively.² For 3C120, which has been variously designated as a Seyfert or N-type galaxy, the apparent separation velocity is 3c (ref. 3). BL Lac has also exhibited qualitatively similar rapid changes in structure. More recent observations indicate that the actual structure might be more complicated than the original simple models but the problem of explaining apparent faster-than-light motions of source components persists.

Classes of models

One might expect apparent faster-than-light expansion motions of relativistically separating components, if the direction of the motion is almost along the observer's line of sight⁵, but such models require somewhat contrived conditions⁶. Moreover, accumulated observations seem to reveal that these apparent faster than light motions are a not uncommon property of compact radio sources, whereas expulsion of components within 10° of the observer's line of sight is improbable.

A second possible explanation of this phenomenon is the "Christmas tree" model in which the radio source is assumed to consist of a number of independently blinking components spread over an extended region⁷.

There are three possible difficulties with this model. First, the luminosities of the blinking components must change such that limitations on the total flux variations from the source are not violated. Second, there should be just as many apparent super-relativistic contractions as expansions in radio sources. Third, in a truly random distribution of blinking components the position angle of the line connecting the two dominant components should also be a function that varies with time; in at least two cases, 3C279 and BL Lac, this position angle seems to be constant^{2,4}. Dent⁸ has answered this objection by suggesting that these random events are occurring in a disc seen edge on but this is improbable if the constancy of position angle is a general property of these sources.

A third possible class of models for sources with rapidly variable structure involves phase velocities of components^{1,9}. The specific model suggested here is of this class.

Particle injection in a strong dipolar field

I assume that the magnetic field of such a source has a dipolar form. The energy and relativistic particle source of the quasar is at the centre of the dipole, and far from this central object¹⁰ the magnetic field lines satisfy the polar coordinate equation

$$r = R_0 \sin^2 \theta \quad (1)$$

Variables are defined as shown in Fig. 1. For simplicity I assume that the observer's line of sight is perpendicular to the axis of the dipole.

At time $t_0 = 0$ a burst of relativistic electrons is emitted by the central object, and I assert that the total energy of these electrons is less than the total magnetic field energy of the dipole. So a train of electrons, the length of which is determined by the duration of the burst, will move out along every field line. The pitch angles of these electrons will be small for two reasons. First, the magnetic field strength drops off rapidly ($\sim r^{-3}$), and the quantity P_{\perp}^2/B (where P_{\perp} is the momentum of the electrons perpendicular to the magnetic field, B) is an adiabatic invariant of the electron motion. Second, electrons with high pitch angle will lose energy rapidly by synchrotron radiation in the central region of high field. Therefore, as the train of electrons travels out along the field lines, their synchrotron radiation will be confined to a narrow cone along the field line, and will not be directed toward the observer. At some time t_1 , however, when the train has reached r_1' (Fig. 1), the 'search light' of the electron train will sweep past the observer, who will see a synchrotron spot at a projected distance d_1 from the central object when the radiation reaches him. Since the dipolar field is symmetric about the equatorial plane, the observer will see two such spots separated by a projected distance of $2d_1$.

At a later time, t_2 , this train of electrons will have moved over the top of the field line and, still emitting synchrotron radiation forwards, will no longer be visible. But a similar train traveling along a higher field line will have reached r_2' at a projected distance of d_2 from the central object. The

two synchrotron spots will now have increased their separation to $2d_2 > 2d_1$. Thus, the radio source will seem to have two components separating with an apparent velocity of

$$V_s = 2(\Delta d)/\Delta t \quad (2)$$

where $\Delta d = d_2 - d_1$. Δt is not the difference in travel times of the two electron trains ($t_2 - t_1$), but the difference in arrival times of the synchrotron radiation from r_2' and r_1' at the observer. Radiation coming from r_2' has less far to travel to reach the observer (by a distance x), so

$$\Delta t = t_2 - t_1 - x/c \quad (2a)$$

where $x = \Delta d \tan \theta$.

This apparent separation velocity (equation (2)) does not represent the motion of two objects; it is a phase velocity of the emission region and is not physically limited by the velocity of light.

For a perfect dipolar field, equation (2) can be solved explicitly to obtain a numerical value for the apparent separation velocity of the synchrotron spots: $V_s = 4.4c$.

This model does not depend on the observer being in the equatorial plane of the dipolar field. In general, one will always observe two separating synchrotron spots regardless of orientation unless the line of sight is coincident with the axis of the dipole, in which case the observer will see an expanding ring. With impulsive injection of electrons, the apparent expansion velocities for orientations other than that described above will be less than $4c$ but greater than $2c$. Further, it is interesting that if particle injection were continuous instead of impulsive, one could see continuous synchrotron radiation along a line corresponding to the projection of the magnetic dipole axis on the plane of the sky—a jet.

Individual source parameters

Several conditions enable estimation of the physical parameters of individual sources. If: (a) the magnetic field is dipolar in form; (b) the energy density of the field is higher than that of the particles; (c) the relativistic particle energy density must be sufficiently great to explain the total radio luminosity up to a frequency of 50 GHz or so; and (d) the pitch angles of the radiating electrons must be less than 10° in the emitting regions; then with the measured separation of the double components in a particular source we may estimate the size (a) of the region over which particle acceleration takes place (that is, the radius of the equivalent current loop producing the dipolar magnetic field), the magnetic field strength in this region (B_0) and the total magnetic field energy (E_H). Assuming cosmological distances (Hubble's constant = 75 km s⁻¹ mpc⁻¹; $q = 1$) I find for 3C120 (ref. 3) $a = 0.3$ pc, $B_0 = 1.0$ gauss; $E_H = 3 \times 10^{53}$

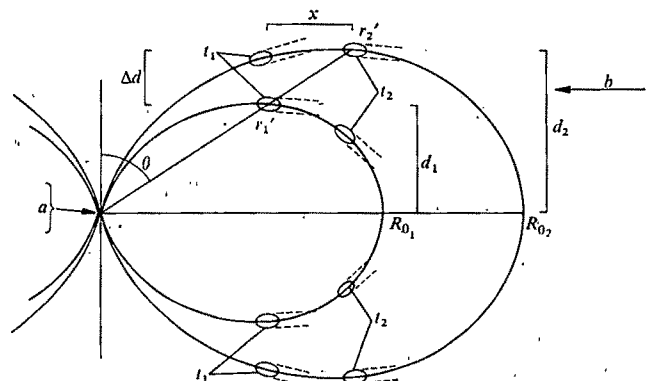


FIG. 1. Dipolar field geometry of a rapid variable structure radio source. *a*, Particle burst injected at $t_0 = 0$; *b*, line of sight.

erg; and for 3C279 (ref. 1) $\alpha = 5$ pc, $B_0 = 0.3$ gauss; $E_H = 3 \times 10^{56}$ erg. The derived field energies are reasonable compared with total energies of fields and particles in quasars and radio galaxies ($\sim 10^{60}$ erg).

For both sources acceleration of electrons takes place over a large region, and it is unlikely that this acceleration occurs simultaneously throughout that region. The maximum separation velocity resulting from impulsive injection of electrons at the centre of a magnetic dipolar field is $4.4c$. But for these two sources the injection cannot be truly impulsive. This offers the possibility of producing arbitrarily high separation velocities of double components. Suppose, for example, that acceleration of particles first occurs near the centre of the equivalent current loop of the extended dipolar field. Trains of electrons moving up the higher field lines of the extended field (Fig. 1) would have a head start on electrons moving up lower field lines. The difference in arrival times of the two electron trains ($t_2 - t_1$) would therefore be decreased and a higher apparent expansion velocity would be observed. Thus the apparent component separation velocity of $6c$ in 3C279 could occur if the injection of electrons at the centre of the extended dipolar field is not impulsive.

Observational predictions

It is difficult to imagine how the orientation of the dipole could change on a time scale shorter than that between successive flares. So I expect that all motions of radio source components should be along the same axis—the axis of the dipole projected onto the plane of the sky. In all subsequent outbursts the apparent motion of the components should be along an axis having the same position angle as the first observed event. This seems to be the case for BL Lac (ref. 4).

Second, since the distribution of electron pitch angles in the synchrotron emission regions (spots) is strongly peaked around zero, I expect a resultant circular polarisation of the

radio emission from the individual source components of the order of a few per cent. The direction of the magnetic field is predominantly toward the observer in one of the spots and away from the direction of the observer in second spot. So the sense of the circular polarisation should be opposite in the two components of the double source. In other words, the relative intensity of the two source components should be different by a few per cent when observed in opposite senses of circular polarisation.

Finally, since the burst of electrons from the central object could be an extended event in time, some sources might have the structure of a double jet with a rapid outward-moving brightening at the two opposite ends of the jets.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Intermittent momentum transport in a geophysical boundary layer

THE development of flow visualisation techniques^{1,2} has prompted a renewal of interest in investigating the dynamics of turbulent boundary layers. The discovery that turbulence is generated intermittently, in "bursts"³, near the wall, is of particular significance. It has also been found that the ejections and inrushes of fluid that accompany the bursts of turbulence generation are dominant contributors to the Reynolds stress⁴⁻⁶. These ejections and inrushes extend well into the flow. It follows that momentum transport in turbulent boundary layers is an intermittent process and that the intermittency is not confined to the region near the wall where most of the turbulence production takes place.

Can such laboratory results on turbulence structure be applied directly to boundary layers of geophysical scale? The potential importance of intermittence-related phenomena in large scale flows has been discussed by Mollo-Christensen⁷

and by Grass⁸. The work by Dorman and Mollo-Christensen⁸ also gives some direct evidence for intermittent momentum exchange in the atmospheric boundary layer.

For some time, a colleague and I have been investigating the turbulent structure of an estuarine, tidal flow and interpreting our results in terms of contemporary research on boundary layers⁹. The continuing analysis of our measurements has included a search for evidence of intermittency in this boundary layer of geophysical dimensions. We have found that the turbulent transport of momentum, or the Reynolds stress, is highly intermittent and that the observations are consistent with the results of wind tunnel and laboratory measurements made at lower Reynolds numbers.

The field experiments were carried out in the Choptank River estuary on Chesapeake Bay. Vertical and horizontal current velocities were sampled every 2 s for approximately two tidal cycles. The overall depth at the measuring station was 8–9 m and currents were recorded at various distances above bottom using a pivoted-vane current meter¹⁰. The maximum tidal flow was approximately 75 cm s^{-1} yielding Reynolds numbers based on depth of the order of 10^6 – 10^7 during most of the cycle. At these flow rates the sampling interval of 2 s allowed detection of turbulence structure with horizontal dimensions ~ 1 m. Turbulent fluctuations in the horizontal current in the direction of the mean flow (u')

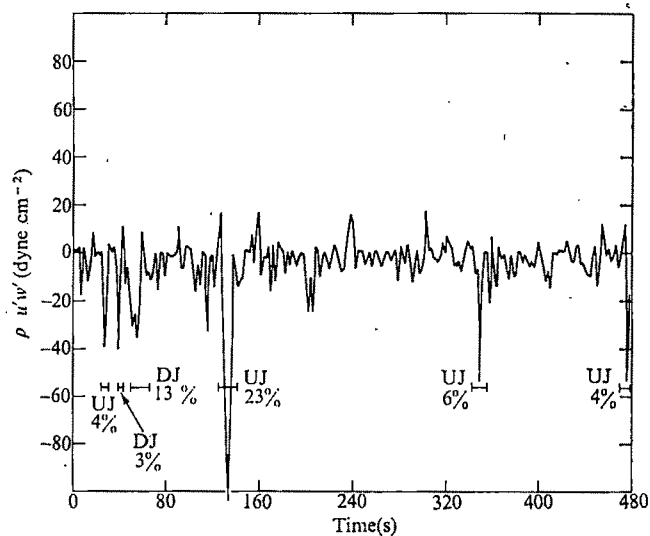


FIG. 1 Time series of the correlation between horizontal (u') and vertical (w') current velocity fluctuations at 2.25 m above bottom showing intermittent, large contributions to the Reynolds stress. Decelerating flood tide (current 62 cm s⁻¹). Run 541-570. $\rho u'w'$ (8 min average) = 5.16. Six events in 7% of the time account for 54% of the measurement of Reynolds stress.

and in the vertical current (w') were obtained by calculating deviations from a third degree polynomial fit to the sampled currents. The velocity fluctuations (u' and w') were then

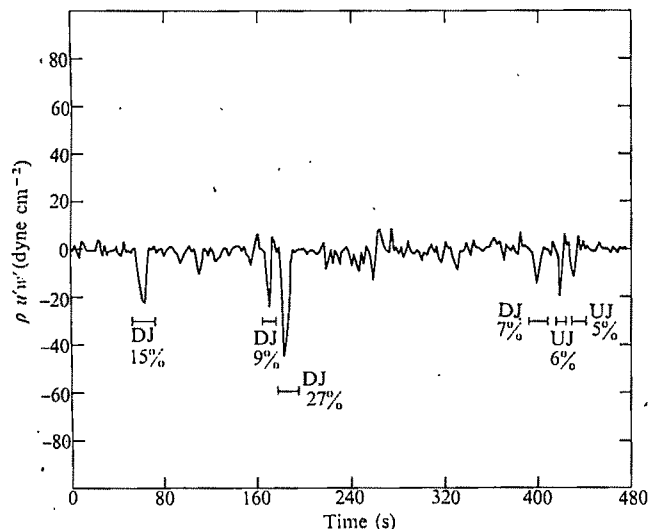


FIG. 2 Time series of the correlation between horizontal (u') and vertical (w') current velocity fluctuations at 1 m above bottom showing intermittent, large contributions to the Reynolds stress. Decelerating ebb tide (current 47 cm s⁻¹). Run 661-690A. $\rho u'w'$ (8 min average) = 1.98. Six events in 9% of the time account for 70% of the measurement of Reynolds stress.

correlated to yield the Reynolds stress component that defines the vertical transport of horizontal momentum, $\tau_{xz} = -\rho u'w'$, where ρ is the density of water. The averaging interval indicated by the bar over $u'w'$ was 8 min. The results presented here represent initial processing of data from 100 such intervals of 8 min taken during different phases of the tide.

Figures 1 and 2 show two typical intervals of 8 min in which individual values of $\rho u'w'$ are plotted against time. The Reynolds stress value is obtained from the average of these velocity correlations. Corrsin¹¹ has cautioned against referring to the individual products $\rho u'w'$ as the "instantaneous

Reynolds stress" but it is clear that there is intermittent structure in these 'events' which, when averaged, make up the Reynolds stress. Much of the final value of τ_{xz} is contributed by a relatively few, intermittent, highly correlated fluid motions. The UJs and DJs classify the kinds of events that transport the momentum—UJ signifies an upward jet of slower moving water ejected away from the bottom boundary into the main stream; DJ indicates a downward jet of faster moving water approaching the bottom. The percentage contributions of each of these events to the Reynolds stress value are shown. Table 1 summarises the relative contributions of events in different quadrants of the $u'w'$ plane to the Reynolds stress. These results are in good agreement with the laboratory data of Wallace, Eckelmann and Brodkey⁶, which were obtained from hot film measurements in an oil channel.

The frequency of momentum transporting events rather arbitrarily depends on the criteria used to define an event. Events in Figs 1 and 2 were simply taken as the six largest values of the products $u'w'$. If any value of $u'w'$ more than two standard deviations from the median is considered to be an event, the data treated so far indicate that events typically occur about every 25 s. This result may be compared with the wind tunnel data of Rao *et al.*¹². Their studies of the "bursting rate" as a function of R_θ (Reynolds number based on the momentum thickness θ) indicate that the period between events scales on the flow parameters of the outer part of the boundary layer, U and δ^* . (U is the mean flow velocity and δ^* is the displacement thickness.) Using the conclusions of Rao *et al.*¹² and typical values of U and δ^* from our data, the predicted period between events is about 35 s. The agreement of the observed and calculated frequency is close enough to indicate that we are dealing with the same kind of intermittent phenomena in the geophysical boundary layer.

Figure 3 consolidates data from 100 samples of 8 min of the type shown in Figs 1 and 2. The abscissa represents the percentage of the time occupied by individual products $u'w'$, selected on the basis of magnitude only, without regard to sign. The ordinate is the percentage contribution that these selected events make to the measurements of Reynolds stress. About 15% of the Reynolds stress is due to events occupying approximately 1% of the time, whereas some 60% of the stress is produced in only 10% of the time. If extrapolated slightly, this result is in good agreement with the wind tunnel observations of Willmarth and Lu⁵ who found that 99% of the stress value was contributed in 55% of the time.

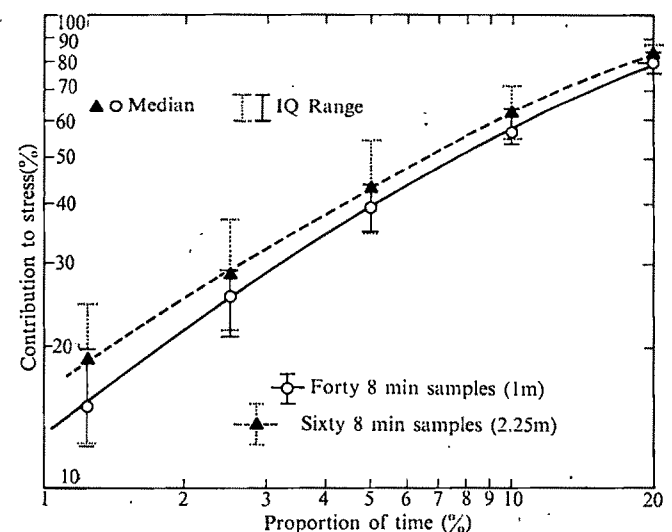


FIG. 3 The degree to which intermittent, momentum transporting events contribute a disproportionate share to the Reynolds stress. IQ, Interquartile.

TABLE 1 Relative contributions of events in the $u'w'$ plane to Reynolds stress (%)

Distance off bottom	$u' < 0, w' > 0$	$u' > 0, w' < 0$	$u' < 0, w' < 0$	$u' > 0, w' > 0$
1 m	65 ± 3	67 ± 6	-15 ± 4	-15 ± 4
2.25 m	73 ± 8	65 ± 8	-17 ± 6	-20 ± 8
Wallace <i>et al.</i> ⁶	~ 68	~ 60	~ -15	~ -15

Our measurements in an estuarine tidal flow indicate that the intermittent phenomena observed in wind tunnel and laboratory boundary layers can be scaled up to dimensions of geophysical interest. It may well be that the intermittence of momentum transport is a prominent and characteristic feature of most geophysical boundary layers.

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"Bursting" phenomena in the sea

INTEREST in the structure of turbulent boundary layers and in particular mechanisms responsible for the transfer of turbulent energy and generation of Reynolds stress has recently been stimulated by a series of laboratory flow visualisation experiments¹⁻⁵. These have indicated that Reynolds stress production is intermittent and associated with a sequence of motions known collectively as "bursting"³. Experimental evidence presented here suggests that similar motions can be readily identified in a geophysical boundary layer at length and time scales unattainable in the laboratory.

The observations reported here form part of a larger programme carried out between 1971 and 1973, in which systematic measurements of near bottom turbulence have been made in the Irish Sea. The primary objective of this study has been to determine by conventional means⁶⁻⁹ the frictional interaction between tidal currents and the sea bed and to extend previous observation^{6,9} of the turbulent structure of bottom boundary layers from estuarine and coastal waters to conditions more typical of circulations in the sea.

Measurements have been carried out at locations shown in Fig. 1, at which depths have varied between 10 and 60 m and maximum surface currents have been of the order of 1 ms^{-1} . The horizontal and vertical turbulent velocity fluctuations u and w have been measured at various heights in the bottom 2 m of the boundary layer using electromagnetic current meters¹⁰. These were mounted on a probe which was lowered to the seabed from the surface. Voltage analogues

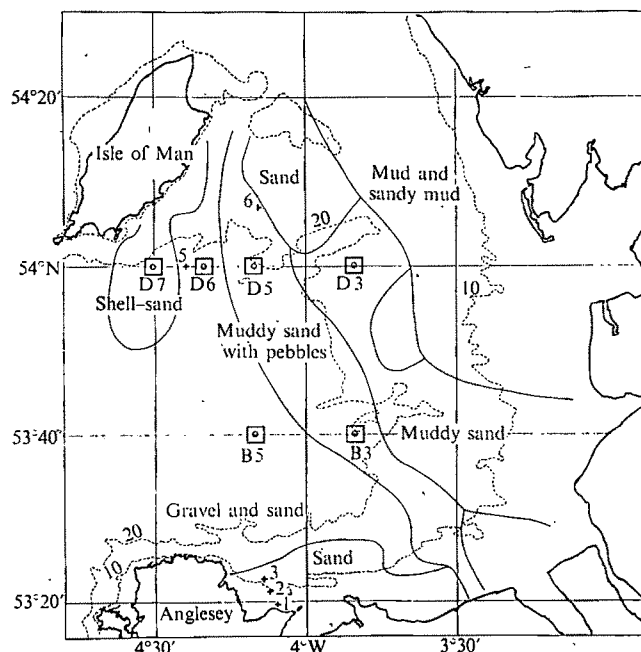


FIG. 1 Locations at which observations of bottom turbulence have been made in the Irish Sea between 1971 and 1973.

of the turbulent velocity components were transmitted to a ship at the surface by cable, recorded on magnetic tape and digitally on punched paper tape. It has been possible to measure simultaneously the contributions to the Reynolds stress ($-\rho u'w'$ where ρ is the fluid density) at two heights above the seabed over a wide range of flow conditions, depth and bottom sediments. Approximately 150 time series (10 min each) of u and w have been obtained, together with measurements of the velocity profile over the entire depth. Records have been replayed through low pass analogue filters into a data logger and digitised at intervals between 0.63 s and 0.08 s. The mean, standard deviation, skewness and kurtosis for each record were calculated before spectral analysis using standard digital methods. The turbulent spectra of u and w and the Reynolds stress cospectrum, corrected for the frequency response of the current meters and filters, non-simultaneous sampling and sensor misalignment, have been computed and are being used to determine those scales of motion contributing to the Reynolds stress.

Reports of bursting phenomena in laboratory experiments¹⁻⁵ have prompted us to examine our records for similar mechanisms. Figures 2 and 3, showing reconstructed analogues of u , w and uw from two different locations, indicate the intermittent nature of the stress input to the boundary layer by a pronounced asymmetry of the uw trace. Record 2/14, made with a sensor of diameter 10 cm and a time constant of approximately 1.0 s, has fewer high frequency components than record 11/4/2, obtained with a sensor of diameter 5 cm having an overall time constant of 0.1 s.

Inspection of the signs and magnitudes of u and w in Fig. 2 identifies the large values in the uw record as belonging to the bursting process and following the usual convention⁵, these events can be classified as follows:

- 1) $u < 0, w > 0, uw < 0$; an ejection of fluid away from the boundary.
- 2) $u > 0, w < 0, uw < 0$; a sweep or inrush of fluid toward the boundary.
- 3) $u > 0, w > 0, uw > 0$; a weak outward interaction of fluid from the boundary.
- 4) $u < 0, w < 0, uw > 0$; a weak inward interaction of fluid toward the boundary.

In particular those terms contributing to the ejection and

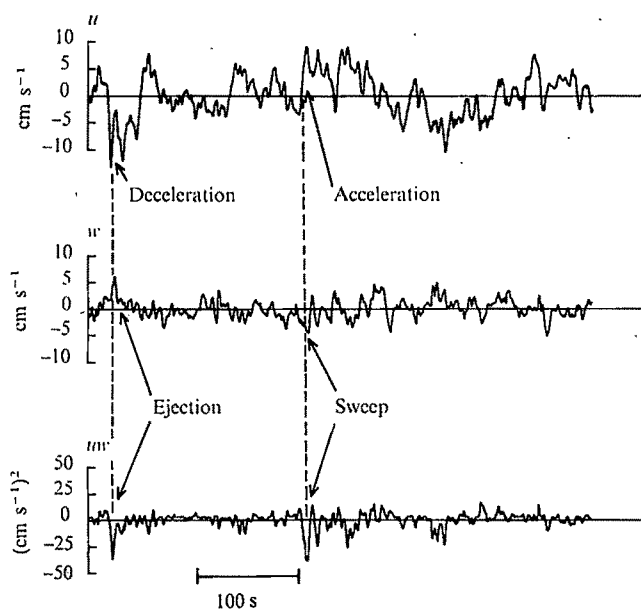


FIG. 2 Bottom turbulence record 2/14, station 2, depth of water 15 m. Analogues of u , w and uw at a height of 1 m above the seabed in a mean current of 22 cm s^{-1} and a Reynolds stress of $2.69 \text{ dynes cm}^{-2}$, showing ejection and sweep phases.

sweep phases have much larger amplitudes than the interaction processes. Comparison of these records with similar laboratory investigations² suggests that the large amplitude events are ejections and sweeps, with an ejection ($w > 0$) preceded by a deceleration of the horizontal flow and a sweep ($w < 0$) occurring after an acceleration.

The distributions of u , w and uw and their departure from gaussian behaviour have been examined in a number of records. The asymmetry of the uw analogue as a result of ejections and sweeps and the net positive input of Reynolds stress, is characterised by a negative skewness of its distribution around the mean. From the distribution of uw in record 2/14 (Fig. 4) it has been possible to determine the contribution of these events to the Reynolds stress. This single record illustrates that events occurring outside -3 standard deviations from the zero stress level contribute as much as 31% in only 3% of the time and events outside -2 standard deviations make a contribution of 57% in 7% of the time. This evidence supports the view¹ that, depend-

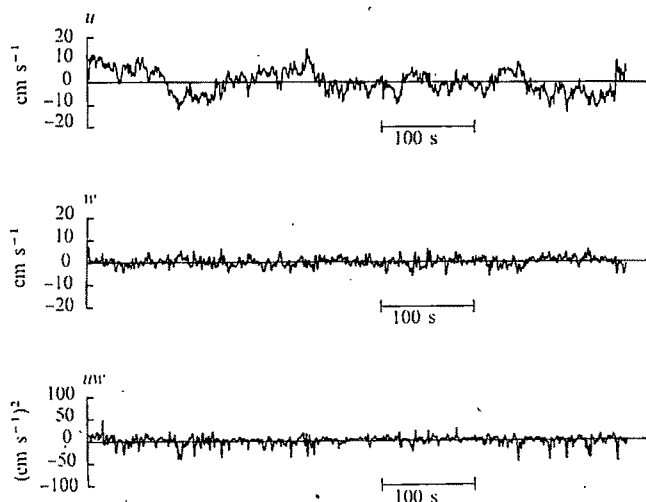


FIG. 3 Bottom turbulence record 11/4/2, station D6, depth of water 40 m. Analogues of u , w and uw at a height of 1.5 m above the seabed for a mean current of approximately 30 cm s^{-1} and Reynolds stress of $3.85 \text{ dynes cm}^{-2}$, showing the intermittent nature of the contributions to uw .

ing on Reynolds Number, as much as 70% of the Reynolds stress may be derived from ejections alone. More recent work⁵ has confirmed that both ejection and sweep motions are more intense than interactions and that in the region of maximum Reynolds stress in a bounded shear flow, ejections are about $1/3$ more intense than sweeps, existing for 20% less time. The duration of ejections and sweeps from our records is of the order of 5 to 10 s with periods between events typically 20 to 100 s. These periods seem to correspond with observed maxima in the Reynolds stress cospectrum⁶.

The implications of bursting phenomena in this example of a naturally occurring boundary layer and their influence on the shear stress at the seabed are not clearly understood. How-

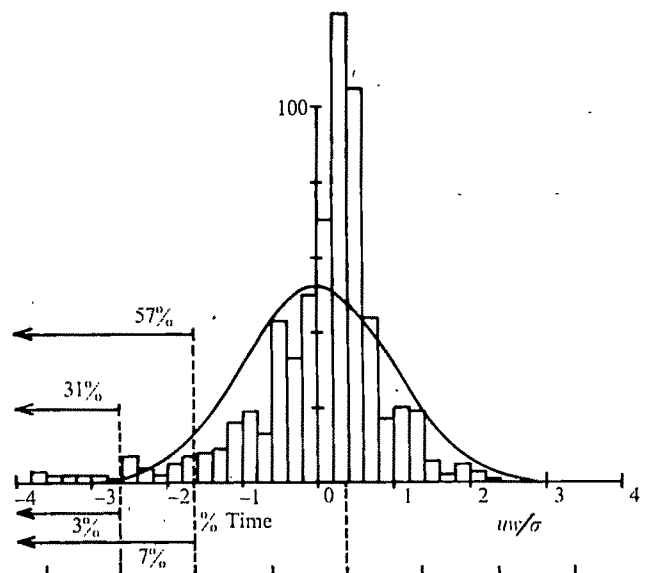


FIG. 4 Distribution of uw in record 2/14 with respect to the mean (upper scale) and zero Reynolds stress (lower scale), showing percentage contributions to uw . Continuous curve is the Gaussian distribution for the same sample and standard deviation. 650 samples; mean $-2.62 \text{ cm}^2 \text{ s}^{-2}$; standard deviation $7.10 \text{ cm}^2 \text{ s}^{-2}$; skewness -1.92 ; kurtosis 9.27 . Percentage contributions to Reynolds stress marked by arrows.

ever, these observations do indicate that the generation of turbulence and Reynolds stress is intermittent and that it is possible to extend the scales of motion involved from laboratory to geophysical proportions.

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Experiment to measure the antimatter content of the Tunguska Meteor

ON the morning of June 30, 1908 a meteor caused great damage in the region of the isolated trading station Vanovara in Siberia, Russia. Investigations of this meteor, called the Tunguska Meteor, have been described by Krinov¹. Light from the meteor was visible even in a sunlit, cloudless sky. There was an explosive wave, with an energy between 3 and 5×10^{23} erg², blowing down trees over an area of approximately 2,000 km². Thermal energy, estimated to be

TABLE 1 Average chemical composition of continental crust weight %

Element	% (weight)
Oxygen	46.6
Silicon	28.9
Aluminium	8.3
Iron	4.8
Calcium	4.1
Potassium	2.4
Sodium	2.3
Magnesium	1.9
Titanium	0.5
Phosphorus	0.1
Manganese	<0.1

between 1 and 2×10^{23} erg, caused fires and seared trees up to 18 km from the centre of the blast. But it appears as though the meteor never reached the ground, for no crater was formed, nor have any fragments been found which can be positively identified as part of the meteor.

Several explanations have been discussed by Krinov¹. Cowan, Atluric and Libby have suggested that it was an antimatter meteor³ and Jackson and Ryan have postulated that it was a black hole⁴.

Cowan *et al.* suggested that if the Tunguska Meteor were a rock of antimatter, nuclear processes associated with the annihilation of the meteor would have increased the amount of ¹⁴C in the atmosphere. Subsequent assimilation of the ¹⁴C by trees might then be detectable. Their analysis of a tree that had been growing in Arizona in 1909 AD showed a smaller increase in the ¹⁴C content than they had predicted. The significance of this was difficult to interpret because of what seemed to be random yearly variations of ¹⁴C in the local atmosphere. Here I again examine the hypothesis of an antimatter meteor, to ascertain whether other experimental tests can be carried out.

Any event involving nuclear processes should produce radioactive nuclei. Of special interest is the electromagnetic cascade shower. This is produced in air by the decay into γ rays of the π^0 s which are produced when antimatter nuclei annihilate. When an electromagnetic cascade shower containing a wide spectrum of γ rays strikes the ground, radioactive nuclei are produced as a result of many different photonuclear processes.

The major constituents of rock and soil are oxygen, silicon and aluminium with smaller amounts of iron, calcium, potassium, magnesium, phosphorous and sodium, in the amounts given in Table 1 (see ref. 5).

To determine which radioactive products might be produced, the reactions (γ, p), (γ, n), (γ, d), and ($\gamma, 2n$) on the various isotopes of the elements listed in Table 1 have been considered. Only a few of these reactions (listed in Table 2) produce nuclei which are sufficiently long lived to be detected now approximately 2×10^9 s after the event.

The reactions which produce ²⁶Al have been given special consideration for two reasons. First, silicon, and to some extent aluminium, are abundant elements in rock (Table 1). Second, ²⁶Al decays predominantly by emitting a positron, whereas ³⁹Ar decays by emitting a β^- ray, and ⁵³Mn by

electron capture. The decay signature of a positron emitter, with the accompanying annihilation radiation, allows the design of systems which will more readily discriminate against background and thus measure smaller total activities.

An experiment to detect the antimatter component of the Tunguska Meteor is thus conceptually simple. The ²⁶Al content of rocks or soil is measured as a function of the distance from the centre of the explosion. The highest concentration of ²⁶Al should be found near the centre. In order to assess the technical feasibility of the experiment, however, the production of ²⁶Al must be examined in more detail.

The number of ²⁶Al atoms, N_{26} , created in the soil and rock by the flux of γ rays is given by the expression

$$N_{26} = \sum_i \frac{A_0}{A_i} f_i \int \frac{dN}{dE} \sigma_i(E) dE \quad (1)$$

where A_0 is Avogadro's constant = 6.02×10^{23} mol⁻¹, A_i is the atomic weight of the nucleus; f_i is the fraction by weight of the nucleus; $\sigma_i(E)$ is the cross section for production for ²⁶Al as a function of photon energy, E , and dN is the number of photons cm⁻² with energy between E and $E + dE$. For the problem under consideration here, equation (1) contains only two terms, corresponding to the nuclei of ²⁷Al and ²⁸Si.

An accurate calculation of the production of ²⁶Al is not possible because of uncertainties in both the incident flux of γ rays and the production cross section of ²⁶Al. Some estimate of these quantities can, however, be made. The γ -ray flux may be estimated in the following way. When a proton and antiproton annihilate, an average of four charged and two neutral pions are produced⁶. Thus about half of the available energy goes into the rest mass of pions and the remainder goes into kinetic energy. Each neutral pion decays into two γ rays which initiate an electromagnetic cascade shower.

The energy density received at the ground is reduced by both the solid angle and absorption in the air. The explosion has been estimated to have occurred between 5 and 6 km up in the atmosphere³. The height of the meteor site is approximately 300 m. At a distance of 5 km, the solid angle subtended by each square centimeter of the ground is 3×10^{-13} sr. The air mass, calculated by using a scale height of 8.4 km and integrating from 0.3 to 5.3 km, is 430 g cm⁻². The energy absorption coefficient of an electromagnetic cascade shower in air is assumed to be the same as that measured for water, that is 0.013 cm² g⁻¹ (ref. 7). This means that approximately 0.37% of the initial energy is transmitted through the air to the ground. The total energy, assumed to be 5×10^{23} erg, or 3×10^{20} MeV, is reduced by a factor of 6 because only a sixth of the energy is converted into γ rays, and by an additional factor of approximately 10^{15} resulting from a combination of the solid angle and absorption. Thus the energy flux at ground level is of the order of 5×10^{13} MeV cm⁻².

The energy spectrum of photons in the cascade shower is uncertain. Because of the kinetic energy of the π^0 s, the initial decay gammas will have a rather broad energy distribution centred at 68 MeV. Processes such as pair production and Compton scattering with subsequent bremsstrahlung by the pair-produced electrons, will further degrade the photon energy spectrum. On the other hand, lower energy photons are more strongly absorbed by the Compton process. (Photoelectric interactions are negligible at the energies considered here.)

Most γ rays produced by cosmic rays and observed in the atmosphere seem to come from π^0 production near the top of the atmosphere⁸. Thus, because the physical process of absorption and creation are similar, the spectrum of the flux induced by the Tunguska Meteor might be expected to be similar to that produced by cosmic rays traversing the same thickness of atmosphere.

If the change in radioactivity is to be detected, the burst of radiation at the Tunguska site must be appreciable when compared with the total cosmic-ray flux integrated over the average lifetime of ^{26}Al . Two estimates of the integrated cosmic-ray flux lead to similar answers. The average radiation background resulting from cosmic rays at sea level is approximately 50 mR yr^{-1} . This implies a total dose of $2.4 \times 10^8 \text{ MeV cm}^{-2} \text{ yr}^{-1}$. The γ -ray flux integrated for energies of about 30 MeV and extrapolated to sea level is approximately $3 \times 10^{-2} \text{ cm}^{-2} \text{ s}^{-1}$ (ref. 8). The integrated energy in this flux yields an energy of $3 \times 10^8 \text{ MeV cm}^{-2} \text{ yr}^{-1}$. The average lifetime of ^{26}Al is $1.07 \times 10^6 \text{ yr}$ and so the additional flux calculated to have been produced by the Tunguska Meteor represents a 15% increase in the total dose received over the past million years.

TABLE 2 Long-lived isotopes

Parent Nucleus	Reaction	Daughter Nucleus	Half-life (yr)
^{27}Al	γ, n	^{26}Al	7.4×10^5
^{28}Si	γ, d	^{26}Al	7.4×10^5
^{41}K	γ, d	^{39}Ar	269
^{54}Fe	γ, p	^{53}Mn	1.1×10^7
^{55}Mn	$\gamma, 2n$	^{53}Mn	1.1×10^7

To obtain the effective production rate, the γ -ray spectrum must be integrated with the cross section as shown in equation (1). The energy spectrum of γ rays in the atmosphere has been measured by Thompson⁸. Very approximately, the energy spectrum of γ rays at a depth of 500 g cm^{-2} can be fit by a power law of the form

$$\frac{dN}{dE} \propto E^{-1.6} \quad (2)$$

The cross sections for the production of ^{26}Al in the ground state by either of the two reactions listed in Table 2 have not been measured. The cross section for $^{27}\text{Al}(\gamma, n)^{26}\text{Al}^*$, where ^{26}Al is the 0.239 MeV first excited state ($\tau_{1/2} = 6 \text{ s}$), has been measured for photon energies up to 62 MeV (ref. 9). If the ^{26}Al is initially formed in a highly excited state, the probability of radioactive decay to the short-lived first excited states with spin and parity of 0^+ should be about the same as for decay to the long lived ground state which has spin and parity of 5^+ . For the predicted spectrum, equation (2), and the measured cross section, the integral evaluated by numerical methods is

$$\int \frac{dN}{dE} \cdot \sigma(E) dE = 3 \times 10^{-15} \text{ photons/nucleus.}$$

Values of the cross section for the second reaction $^{28}\text{Si}(\gamma, d)^{26}\text{Al}$ have not been reported. The cross section for $^{32}\text{S}(\gamma, d)^{30}\text{P}$ has, however, been measured from threshold to 80 MeV (ref. 10). If this measured cross section is used, a numerical value of the integral is obtained.

$$\int \frac{dN}{dE} \cdot \sigma(E) dE = 2 \times 10^{-15} \text{ photons/nucleus}$$

In the following discussion a value of 2×10^{-15} photons/nucleus is adopted for both reactions.

The amounts by weight of silicon and aluminium in average rock are 29 and 8%, respectively (Table 1). The production of ^{26}Al in rock at the centre of the meteor site, is therefore estimated to be 4×10^8 atoms per kg of rock. With an ^{26}Al half life of 7.4×10^5 years, this corresponds to an induced specific activity of approximately $10^{-2} \text{ d.p.m. kg}^{-1} \text{ rock}$.

Tanaka *et al.*¹¹ have attempted to measure low levels of activity due to ^{26}Al found in deeply buried rock. They report an upper limit of $10^{-2} \text{ d.p.m. kg}^{-1} \text{ rock}$, a value

which is approximately the size of the predicted effect.

Most detection systems for measuring very low levels of positron emission consist, at least in part, of opposing NaI(Tl) crystals which detect in coincidence the two 0.511 MeV γ rays from the annihilation of the positron^{12,13}. For such a system, and for a given observing time, the sensitivity, S , is defined as the ability of a detector to measure activity, and

$$S \propto \frac{\epsilon N}{\sqrt{B}} \quad (3)$$

where ϵ is the detector efficiency, N is the number of nuclei that can be placed in the detector and B is the background rate. Tanaka *et al.*¹¹ suggested that the sensitivity of their instrument could be improved if larger detection crystals were used and if purer and more concentrated samples were obtained. These ideas suggest that considerable increase in sensitivity should be possible. For example, NaI(Tl) crystals are available with an area up to 100 times that of detectors used by Tanaka *et al.*¹² and Roedel¹³, and three times as thick. Even if the background increases linearly with the volume of detector, sensitivity would be increased by a factor of six because more sample could be placed between the crystals. In addition, such large NaI(Tl) would increase the photo-fraction efficiency and thus the sensitivity.

The use of pure aluminium, rather than the Al_2O_3 used by Tanaka *et al.* would increase the reported sensitivity by a factor of about two because more Al nuclei could be introduced into the same volume.

It might be possible to select rocks, such as quartz or limestone; which are largely free from aluminium. The extraction of aluminium from such rock samples would increase the specific activity of ^{26}Al in the sample by more than an order of magnitude.

In addition to the ideas discussed above, a promising method of reducing the background in β^+ counting systems, by observing the positron in coincidence with the annihilation γ rays, has been developed¹⁴. We have recently developed a simple version of such a system which yields a gain in sensitivity of a factor of two over an equivalent two- γ -ray detector system.

With the technical improvements in detectors that seem feasible, a system with an increase in sensitivity of almost two orders of magnitude could be planned. This would allow the detection of an increase of only a few % in ^{26}Al content. The proposed experiment could then be readily carried out.

I acknowledge the impetus of a lecture by Professor C. L. Cowan. I also profited greatly from conversations with many colleagues and especially C. L. Cowan, C. J. Crannell, C. Fichtel, J. M. Finn and D. J. Thompson. This work was supported in part by a National Science Foundation grant.

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Comets, solar wind and the D/H ratio

I wish to speculate on the consequences of considering together two astronomical observations. First, the Sun emits a matter flux of $\sim 10^{12}$ g s⁻¹ in the form of a solar wind¹. The chemical composition seems to be rather similar to the solar photospheric composition². So it incorporates a fractional mass of about 1% of atoms which are good candidates for grain formation (ices as CH₄, NH₃, H₂O; dusts as silicates and irons). Thus a flux of $\sim 10^{10}$ g s⁻¹ of condensable matter flows away in the remote parts of the Solar System.

Second, the number of newly arriving comets (of long period) is about one a year with a mean mass of $\sim 10^{18}$ g (ref. 3) and a comet can be pictured plausibly as a conglomerate of ices and dusts⁴. Thus a mean flux of $\sim 3 \times 10^{10}$ g⁻¹ of cometary matter flows inwards from the remote part of the Solar System.

These fluxes seem to agree within one order of magnitude or better. I assume that this is not a pure coincidence.

The process could be pictured in the following way. The ionised atoms of the solar wind follow the laminar flow lines until they reach a boundary where, presumably because of interaction with the interstellar magnetic field, the wind becomes turbulent. There, they are progressively decelerated, recombine, form molecules and eventually grains of increasing sizes. (Although very little is known about how the grains are formed, observations of interstellar matter indicate that the process must be very efficient; otherwise, how could the striking depletion of the refractory elements in the interstellar gas^{5,6} be explained?) The grains are carried away by the turbulent gas and collide with each others. Dust and ices will mix and stick together, thus generating the embryos of comets.

These objects are subjected to two forces: the inward gravitational pull of the Sun and the drag force of the turbulent motions with its residual outward component. As the size of the object increases the second force becomes gradually weaker than the first. The outward motion is decelerated until the object start moving toward the Sun. A comet is born.

So we have a picture of a stationary cloud in which matter is fed in through the solar wind, and out through conglomerates of ices and dusts. The cloud has, in the past, grown to a stage at which the feeding out just compensates the feeding in, thereby explaining the rough equality between the two fluxes.

Before this model becomes credible several points will have to be clarified, several quantitative estimates (of the location and properties of the cloud, of the accretion rate in the cloud and so on) will have to be carried out and several 'tests' will have to be 'passed.' For example, the observed distribution of kinetic energies, of ellipticities and of orbital inclinations of the comets should find a reasonable explanation within the frame of the model.

The orbital planes of the long-period comets do not seem to raise any problem: they are tilted in all directions as expected from the plausible spherical symmetry of the solar

wind. The eccentricities would be assigned to such effects as the random drag velocities of the cells.

But at present, instead of pushing this discussion further, I stress the importance of measuring the D/H ratio in cometary matter by showing its relevance to the problem of the origin of the comets.

The D/H ratio in interstellar space is 1.5×10^{-5} (ref. 7). In oceanic and meteoritic water, on the other hand, the ratio is 1.4×10^{-4} ; the increase is probably due to molecular exchange reaction at the time of formation of the water⁸. In the solar wind D/H $< 3 \times 10^{-6}$, (ref. 9) compatible with the fact that the Sun burned its deuterium in its early days.

Clearly this model of comet formation would predict essentially no D (D/H $\ll 10^{-5}$). If, on the other hand, the comets were made early in the history of the Solar System, by matter left over from the protosolar condensation (the Oort's cloud), depending on their formation temperature we should have in the various cometary molecules a D/H ratio of anything from 10^{-5} to 10^{-4} and possibly even more (see tables in ref. 10). Measurements of DCN/HCN, CH₃D/CH₄, OD/OH in comets would be of the utmost importance.

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Evolution of dense galactic nuclei through dwarf star collisions

STELLAR collisions are important in dense galactic nuclei¹⁻³. QSOs, N galaxies and Seyfert galaxies release energy on similar short time scales⁴⁻⁷ but detailed models of stellar collisions have been limited so far to collisions between two main sequence stars, with the conclusion that for an initial relative velocity $V_{rel} \lesssim 10^3$ km s⁻¹, and small impact parameter, coalescence follows. At $V_{rel} \sim 5,000$ km s⁻¹ two colliding main sequence stars are largely disrupted and a non-negligible amount of relativistic particles is formed⁸. Here we draw attention to the fact that a very important type of collision in dense galactic nuclei is one in which a white dwarf (WD) is involved.

Van den Bergh⁹ has pointed out that most collisions in the nuclei of 'normal' galaxies such as M32 involve two dwarf M stars or one M dwarf (dM) and one late-type giant. The reason is the high abundance of dM stars and the large cross section of giants. The evolution of a nucleus therefore depends on the outcome of WD-WD, WD-dM, dM-dM and dM-giant collisions. We neglect dM-giant collisions because most of them will occur in the highly rarified atmosphere of a giant and consequently will have a very small effect. For simplicity we discuss only dM-WD collisions with impact parameter p smaller than $0.75R$, where R is the radius of the red dwarf.

The escape velocity of a white dwarf varies from 2,500 km s⁻¹ for a WD with $M = 0.25M_{\odot}$ to 16×10^3 km s⁻¹ for a WD with $M = 1.25M_{\odot}$; it is ~ 600 km s⁻¹ for late-type main sequence stars. Consequently, the WD-dM collision of low V_{rel} is governed by the white dwarf gravitational field. As the initial relative velocity of the colliding stars increases (the stellar cluster becomes denser) the dynamics of the collision are governed more and more by the initial relative velocity.

We consider a typical collision between a WD of $1M_{\odot}$ and a dM star of $0.2M_{\odot}$ at $V_{rel} \sim 10^4$ km s⁻¹ and impact parameter $p < 0.75 R$. Since this velocity is greater than the speed of sound in the dM star the effect of gravity on the structure of the red dwarf is small. Further, the change in the velocity of the WD during the collision can be neglected. The time spent by the WD inside the dM star is 10 to 30 s which is more than an order of magnitude smaller than the dynamical time scale of the dM star. So only a small amount of mass can be accreted onto the WD from the dM star. Hoyle and Lyttleton⁹ found that the "capture radius" σ of a star of mass M moving at constant velocity V through an infinite medium is $\sigma = 2GM/V^2$, where G is the gravitational constant. In our example $\sigma \sim 3 \times 10^8$ cm and the density scale height h_p for the dM is $\sim 10^9$ cm for $r \lesssim 0.75R$. So the assumption of an infinite medium is valid. But σ is about $\frac{1}{2}$ the radius of the WD and Hoyle and Lyttleton's theory is not valid for $\sigma < R_{WD}$. A plausible assumption is that $\sigma \sim R_{WD}$ and the total mass accreted by the WD is then $(1 \text{ to } 3) \times 10^{-4}M_{\odot}$. All other parameters being equal, the total mass accreted by a WD will increase for white dwarfs with lower mass and larger radius.

Most of the accreted material is rich in hydrogen because nuclear timescales in a $0.2M_{\odot}$ star are longer than the Hubble time. When the mass accreted exceeds $\sim 10^{-5}M_{\odot}$ nuclear energy generation becomes violent enough to drive a thermal runaway¹⁰⁻¹³ typical of novae. The fate of the runaway depends on the WD initial temperature T (ref. 13). At $T \lesssim 3 \times 10^7$ K the runaway ends in an explosion whereas in hot white dwarfs with $T \gtrsim 4 \times 10^7$ K the energy generation rises until a steady state is reached. At some later time the hydrogen is exhausted and the nuclear reaction decays. In models calculated by Saslaw²³ 10^{47} to 10^{48} erg is released. It will be shown later that plausible models of galactic nuclei give time intervals, between subsequent collisions, of 10^6 to 10^7 yr for each white dwarf. Since the cooling time of white dwarfs is $\sim 10^9$ yr most white dwarfs have $T > 4 \times 10^7$ K and the second process mentioned above occurs. The typical white dwarf thus produces a series of novae at a rate of one per 10^6 to 10^7 yr.

Similar arguments concerning collision energetics show that most dM-dM collisions lead to substantial mass loss if $V_{rel} \sim 10^4$ km s⁻¹, and that the rarer head-on collisions disrupt both stars. Thus, dM-dM collisions transform the dM stars into white dwarfs made of unburnt nuclear material.

In the case of WD-WD collisions a simple estimate yields a collision cross section $\sigma_{WD-WD} \sim (500)^{-1} \sigma_{WD-dM}$ for $V_{rel} \sim 10^4$ km s⁻¹. At $V_{rel} \sim 10^4$ km s⁻¹ the most probable outcome of a collision of two white dwarfs of $1M_{\odot}$ is one white dwarf with a total mass in the range 1 to $2M_{\odot}$. Such a velocity is too low to be energetically capable of disrupting the stars. The WD-WD collisions differ from the WD-dM collisions in two respects. First, WD-WD collisions raise the mass of the remaining white dwarf more than a WD-dM collision and may bring the final product to a mass above the Chandrasekhar limit. Second, the frequency of WD-WD collisions is two or three orders of magnitude smaller than the frequency of dM-WD collisions. This will probably give rise to supernova explosions of a certain frequency.

The evolution of a dense galactic nucleus depends on the transformations between the various stellar species due to stellar collisions. We consider a highly simplified model of a

spherical galactic nucleus of radius R made up of N_{dM} dM stars of $0.2M_{\odot}$ and radius $r_{dM} \sim 0.2R_{\odot}$, and N_{WD} white dwarfs of $1M_{\odot}$ and radius $r_{WD} \sim 8 \times 10^{-3}R_{\odot}$.

We assume that the average velocity can be obtained from the Virial Theorem. The mean free path of a white dwarf for collision with a dM star is

$$\lambda_{WD-dM} \sim \frac{R^3}{N_{dM} r_{dM}^2}$$

and similar expressions hold for λ_{dM-dM} and λ_{WD-WD} . The total number of WD-dM collisions per unit time, n_{WD-dM} is given by

$$n_{WD-dM} \sim 3 \times 10^{18} N_{WD} N_{dM} M_i^{1/2} R^{-7/2} \text{ s}^{-1}$$

where M_i is the total mass of the nucleus and we assume roughly equal velocities for all stars.

We consider the following ranges for the various parameters: $N_{dM} = (0.2 \text{ to } 20) \times 10^{10}$, $N_{WD} = (0.2 \text{ to } 20) \times 10^{10}$ and $R = (0.25 \text{ to } 1.0)$ pc (see refs 14 and 15) in which the parameters of NGC4151 are discussed). Two fairly extreme cases are $R = 0.25$ pc, $N_{dM} = 2 \times 10^{10}$ and $N_{WD} = 2 \times 10^{11}$; and $R = (0.25 \text{ to } 1.0)$ pc (see refs 14 and 15 in which the typical energy releases for novae are $\sim 10^{47}$ erg then $\sim 10^{48}$ erg s⁻¹ and $\sim 10^{42}$ erg s⁻¹ are released in these two models. The supernova rate varies from 10^3 yr⁻¹ to 10^{-3} yr, respectively. But most of the contribution to the total energy release does not come from the supernovae.

Eventually, the number of dM stars decreases and with it the total energy output. If one starts with $N_{dM} \sim 2 \times 10^{11}$ (all of $\sim 0.2M_{\odot}$) and $N_{WD} \sim 2 \times 10^{10}$ (all of about $1M_{\odot}$) the energy output matches that of bright QSOs. A reduction of N_{dM} by a factor of 10 (which is caused by dM-dM collisions over a timescale of 10^6 yr) brings to total energy output to the range of N-type galaxies. Still another reduction by a factor of 10 (over a timescale of 10^8 yr) reduces the energy output to the range of Seyfert galaxy nuclei. Finally, in about 10^9 yr N_{dM} drops to $\sim 2 \times 10^9$ and the energy output due to collisions falls to about 10^{40} erg s⁻¹. The timescale for the change in N_{dM} is easily obtained from the collision frequency and average mass loss per collision. Clearly, the timescale for changes becomes longer as the number of dM stars decreases. At the same time the principal energy source changes as well. At early stages it is predominantly the dM-dM collisions whereas later it becomes the WD-WD collisions and finally the WD-dM collisions.

So it seems that, assuming about 10^{47} erg to be released in a nova caused by a WD-dM collision, we find that stellar collisions in dense galactic nuclei can explain the basic energetics of QSOs, N-type galaxies, Seyfert galaxy nuclei and the central regions of bright elliptical galaxies, as well as their evolutionary connection and sequence.

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Observation of $O^+(^2P^o-^2D^o)\lambda 7,319\text{\AA}$ emissions in the twilight and night airglow

TEN years ago Dalgarno and McElroy¹ suggested that the $^2P^o \rightarrow ^2D^o$ transition of the atomic oxygen ion would be produced in the upper atmosphere through the simultaneous photoionisation and excitation of atomic oxygen atoms by solar ultraviolet radiation.

The photoionisation cross section calculations of Dalgarno, Henry and Stewart² indicate that approximately 25% of the photons absorbed by atomic oxygen below 663 Å will produce O^+ ions in the metastable $^2P^o$ state. Using these cross sections, Dalgarno and McElroy³ computed dayglow emission rates and later extended their calculations to the twilight situation⁴. This excitation mechanism has been observed in the laboratory in photoelectron spectroscopy measurements by Jonathan *et al.*⁵ who found an efficiency of ~22% for producing the $^2P^o$ state at 584 Å. Thus, both theory and experiment indicate that photoionisation excitation of $O^+(^2P^o)$ is an efficient process and should produce an observable $^2P^o \rightarrow ^2D^o$ airglow feature as predicted in the work of Dalgarno and McElroy.

As yet no observations of this transition in the airglow have been reported.

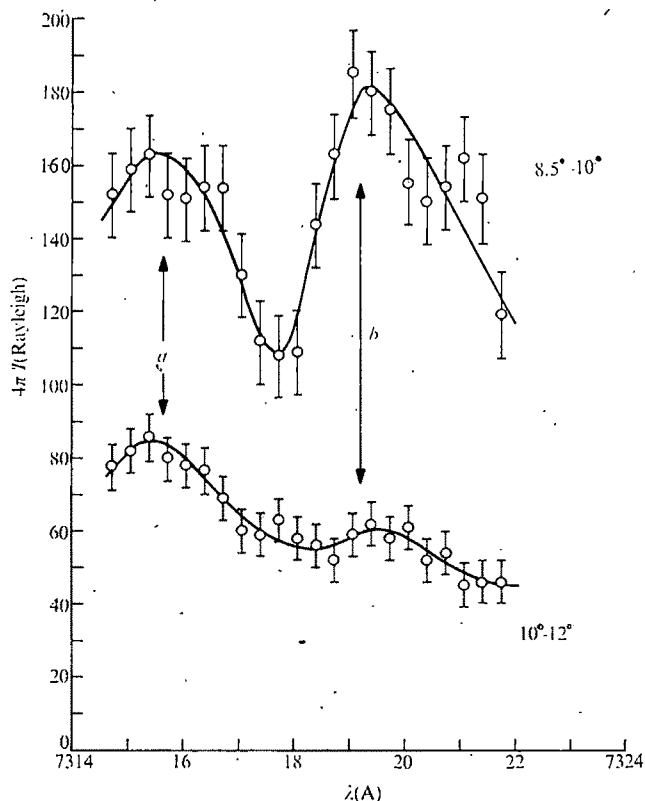


Fig. 1 Upper spectrum: average of the observations obtained during evening twilight for solar depression angles in the range 8.5° to 10°. Lower spectrum: evening scans between 10° and 12°. Points shown represent three-point running averages. a, OH (8-3) $P_1(2)$ feature; b, $O II (^2P^o-^2D^o)$ feature. August 26-30, 1973.

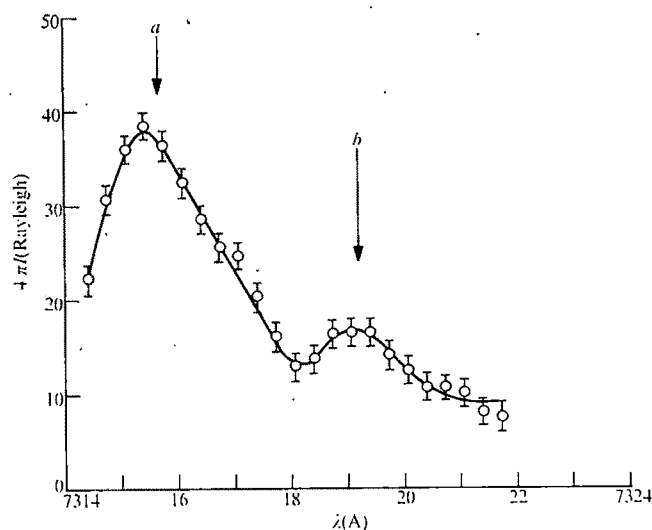


Fig. 2 Observed night airglow spectrum. a, b as Fig. 1. August 26-30, 1973.

This work reports twilight and nightglow observations of the $^2P^o_{1/2, 3/2} \rightarrow ^2D^o_{5/2}$ line group at $\lambda 7,319\text{ Å}$. Assuming that the upper J levels are populated according to their statistical weights, then the $^2P^o_{3/2} \rightarrow ^2D^o_{5/2}$ $\lambda 7,319.4\text{ Å}$ line will be stronger by approximately a factor of three or greater than the $^2P^o_{1/2} \rightarrow ^2D^o_{5/2}$ $\lambda 7,318.6\text{ Å}$ line. The mean wavelength for the two lines is then approximately $7,319.2\text{ Å}$. The observations were made in the zenith at Table Mountain which is ~70 km NNE of Los Angeles and 2,300 m above sea level. The sky conditions there are excellent for airglow observations. A 0.3 m $f/5.3$ spectrometer with a 1,200 lines mm^{-1} grating blazed at 5,000 Å was used to disperse the spectrum. The entrance and exit slit sizes were $55\mu\text{m} \times 2\text{ cm}$ and $65\mu\text{m} \times 2\text{ cm}$ respectively, which resulted in a spectral resolution of approximately 2.3 Å. Any second order contribution was reduced using two interference filters, one in reflection and one in transmission. The radiation was detected with an EMI 9558 QB photomultiplier with an S-20 photocathode cooled to -20°C . Using magnetic and optical lenses, only the central portion of the photocathode was effective, resulting in a dark counting rate of 1-2 s^{-1} . The observations were obtained during the period of new Moon, August 26-30, 1973. The wavelength region 7,314 to 7,322 Å was scanned repeatedly at 5 Å min^{-1} during twilight and accumulations for 4 s were recorded. The nightglow measurements were obtained at slower scanning speeds and longer integration periods.

The Rayleigh scattering component of the twilight was estimated by least-squares fitting and extrapolation of the signals observed for solar depression angles between 4° and 8°. This contribution was subtracted from the observed signals, with the dark count signal, to give the spectra shown in Fig. 1.

It is clear from Fig. 1 that the $O^+(^2P^o-^2D^o)$ $\lambda 7,319\text{ Å}$ feature is present in the twilight spectrum and decreases in intensity as the solar depression angle increases, in qualitative agreement with the variation expected for excitation by solar ultraviolet radiation. The absolute intensity of the O^+ feature is greater than that predicted by Dalgarno and McElroy. This may be due to both experimental error and differences between the actual and model atmosphere and the absorption cross sections used in the calculations⁴. The nearby OH(8-3) $P_1(2)$ line⁶ is also apparent and seen to diminish in brightness as evening twilight progresses approximately as predicted by the model of Shimizaki and Laird⁷.

In the nightglow, where direct solar excitation is absent, the O^+ feature is still found (Fig. 2). We suggest that extreme ultraviolet photoionisation excitation is also responsible for

at least part of this nightglow feature. Both He I $\lambda 584 \text{ \AA}$ and He II $\lambda 303 \text{ \AA}$ emissions will be incident on the night-time upper atmosphere, arising from resonance scattering of the solar helium lines, the former by interplanetary and exospheric He and the latter by plasmaspheric He^+ ions⁸⁻¹². It is difficult to estimate the nightglow $\lambda 7,319 \text{ \AA}$ intensity that would be produced by this excitation mechanism since the background ultraviolet radiation can show considerable variation. The He I $\lambda 584 \text{ \AA}$ may exhibit a seasonal variation due to the non-uniform interplanetary density distribution¹³ and plasmaspheric He^+ concentrations, and the resulting $\lambda 303 \text{ \AA}$ flux, can also show large fluctuations.

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An organic 'metal'?

LITTLE¹ has proposed a mechanism by which an electron-electron (e-e) interaction in certain organic polymers and macromolecules might result in a high superconducting transition temperature. This e-e mechanism requires a combination of two systems consisting of a conducting or semiconducting polymeric compound forming a spine, with highly polarisable side chains attached to the spine. According to Little, the addition of such side chains can increase the e-e attraction in the spine to the point where it becomes energetically favourable to enter the superconducting state. This e-e mechanism of superconductivity has led to lively discussions, primarily concerned with superconductivity in quasi one and two-dimensional systems^{2,3}, and the magnitude of the e-e attraction^{4,5}. It has been shown theoretically³ that, depending on the nature of the interaction between the electrons, a quasi one-dimensional system may exist in different states: metallic, dielectric, antiferromagnetic and superconducting. It has also been shown experimentally that the organic charge transfer salt N-methyl-pehnazinium tetracyanoquinodimethan has 'metallic' properties at temperatures above 200 K with a continuous transition to a small-band-gap insulator below 200 K (ref. 6).

We have been investigating a number of organic compounds in which the e-e interaction is expected to be large. Here we wish to report one of a potentially large class of organic compounds which has 'metallic' electrical properties over a large temperature range. This compound, we believe, is an intercalation compound consisting of a triphenylmethane dye (malachite green) and graphite. Graphite in which the bonding between the carbon layers is weak has been known to form intercalation compounds with alkali metals⁷, acids⁸, halogens⁹ and metal chlorides⁹. The intercalation compounds of K, Rb, and Cs with graphite have been shown to have superconducting transition temperatures of 0.55, 0.155, and 0.135 K respectively¹⁰. (Metallic conduction has also been observed in the metal chloride intercalation compounds⁹.) In the complex described here the graphite, which is a highly conjugated layer compound, acts as the semiconducting spine or sheet, and the dye, complexed to the graphite, is similar to the polarisable side chains of Little's model. The chemical structure of the malachite green dye molecule, and two of its resonance structures is shown in Fig. 1.

This complex, and others of the same general class, is prepared as follows: very high purity samples, 98-99% pure, of malachite green dye and powdered graphite are thoroughly mixed in a mass ratio of three parts of dye to one part of graphite. The powder mixture is heated to the melting point of the dye. The melting point of the dye depends on the purity of the sample and is, in general, greater than 250° C. When the entire sample is in a soft to semi-molten state, the complex is allowed to cool to room temperature when it becomes a hard, black vitreous solid.

Powder X-ray diffraction studies of this compound have shown a broad diffraction peak equivalent to a lattice spacing of 6 Å which may indicate the formation of an intercalation complex. Probes for resistance measurements were usually inserted into the complex while in the soft state, at 150-200° C and the complex was allowed to cool. On cooling, the probes became tightly bound to the sample. Resistance measurements were usually made with copper probes, 1 cm long, separated by distances of 0.4 cm.

Figure 2 is a plot of resistance against temperature for the dye-graphite complex between 77 and 333 K. Between 77 and 250 K, the resistance is seen to be proportional to the temperature, with a slope of 0.1 ΩK^{-1} . At temperatures above the softening point (40 C-60° C), the curve again becomes linear, with a slope of 22 ΩK^{-1} . Furthermore, four-probe resistivity measurements have shown the resistivity to have the same dependence on temperature as the resistance. Unlike most organic complexes based on Little's model¹¹, this complex is rather unique in that over the temperature ranges investigated, there seems to be no activation energy. Furthermore, between 77 K and 250 K the proportional relationship between resistance and temperature is not unlike that for metals at temperatures greater than the Debye temperature. It should be stressed that this complex does not necessarily form a stoichiometric ratio. Resistance measurements indicate that the graphite is gradually converted from a semiconducting state to a 'metallic' state by

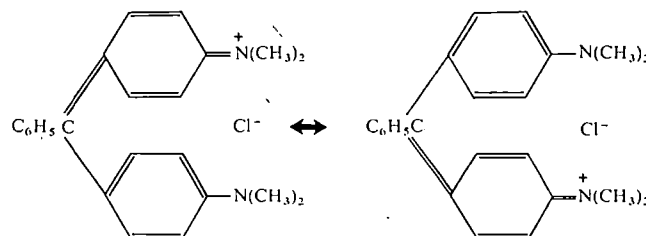


Fig. 1 Molecular structure of malachite green dye showing the two resonance structures which correspond to a positive formal charge on the two amine groups.

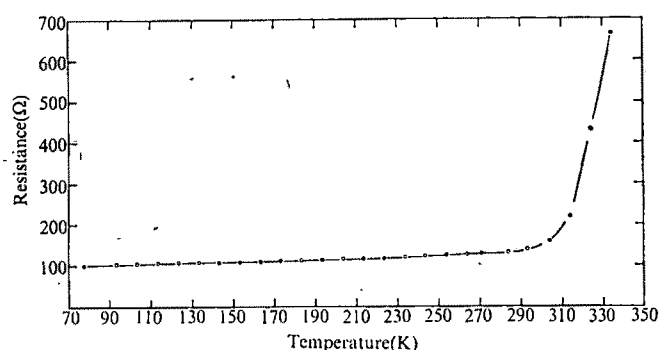


Fig. 2 Plot of resistance against temperature for malachite green dye-graphite complex.

increasing dye concentrations. Further increased dye concentrations convert the graphite back to the semiconducting state.

If the conductive properties of this complex result from an e-e interaction, the fact that it does not exhibit a 'high' temperature superconducting state, if a superconducting state at all, is an extremely complicated problem. Malachite green has an absorption band in the region 600–700 nm. Calculations¹² show that it is necessary to have more polarisable dyes, with absorption bands in the region of $\sim 1,200$ nm, to induce a sufficient e-e interaction in the spine. Furthermore, it is expected that the e-e interaction depends strongly on the distance separating the dye and the spine. For large separations, the interaction decreases rapidly¹² whereas for short separations (strong interaction) exchange interactions can occur between the conduction electrons in the spine and the dye molecule. Such exchange interactions could limit the strength of the e-e interaction in the spine, by the conversion of the dye to a 'stable', less polarisable radical. Nevertheless, if further measurements confirm the 'metallic' state of this complex, or class of complexes, it would indicate that the Coulomb interaction between localised electrons in the conjugated graphite sheet is greatly reduced. Such screening of the Coulomb interaction by the dye would then allow electron delocalisation to the extent that a 'metallic' state results.

We believe that this complex is only one of a rather large class of similar compounds based on the intercalation of triphenylmethane dyes (general class) and 'layer type' semiconductors and conductors. Examples of such 'layer type' semiconductors include graphite, MoS₂, and BN; whereas, TaS₂ and WS₂ are examples of 'metallic' layer compounds. Results of further detailed investigations concerning these compounds in the polycrystalline and single-crystal state will be published elsewhere.

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Rotation of the geomagnetic field

THE geomagnetic field apparently drifts westward about the geographic axis at about $0.2^\circ \text{ yr}^{-1}$. Malin & Saunders have attempted to determine the directions of the axes about which a 'westward' drift would best fit the fields during six successive epochs¹. They did not determine the circular standard deviations of the axis directions but, as the five successive axes moved fairly smoothly through an arc of about 20° , they assumed that the axes had been reasonably well determined, and interpreted the movement in terms of physical processes in the Earth's core.

The axes they determined were, however, almost certainly not those appropriate to their interpretation, and it is unlikely that the correct axes can be determined to a useful accuracy.

They made a numerical search for that combination of axis and drift which maximised the correlation between a function f , specified on the surface of a sphere at one epoch, and the corresponding function F five years later; that is, they looked for that combination which maximised the mean value of f^*F over the sphere, where f^* is the rotated version of f . This process is in fact exactly the same as searching for that combination of axis and drift which minimises the mean square of the difference $(F - f^*)$, a problem in least squares. This, in turn, is exactly the same as fitting the difference $\Delta F \equiv (F - f)$ by the difference $\delta f \equiv (f^* - f)$ by least squares, that is, fitting the integrated secular variation by the 'westward' drift of the field.

The functions f and F were specified in terms of a finite series of coefficients, g_n^m , h_n^m and G_n^m , H_n^m , for $n = 2$ to 6, of (semi-normalised) spherical harmonics defined with respect to the geographic axis. Correspondingly ΔF can be specified in terms of coefficients ΔG_n^m , ΔH_n^m , and, after some manipulation, δf in terms of δg_n^m , δh_n^m . Because the spherical harmonics are orthogonal over the sphere, minimising the mean of $(\Delta F - \delta f)^2$, is the same as minimising the sum

$$S = \sum_n \alpha_n \sum_{m=0}^n [(\Delta G_n^m - \delta g_n^m)^2 + (\Delta H_n^m - \delta h_n^m)^2], \quad (1)$$

where α_n is the mean square value over the sphere of the function produced by a unit amplitude harmonic n , m ; because of the normalisation, α_n is independent of m .

The axis of drift may be obtained from the geographical axis by a rotation having components $(u, v, 0)$ in the $\lambda = 0^\circ$, $\lambda = 90^\circ$, and $\theta = 0^\circ$ axes respectively, and the westward drift rate about the new axis may be denoted w . For convenience the westward drift $[(u, v, 0); w\delta t]$ may be denoted by Ω .

To perform the rotation of axes, Malin and Saunders used the Taylor series method of James². As u and v were at most 14° , and $w\delta t$ was about 1° , it is sufficient to take the series to second order for the following discussion. This shows that, for example,

$$\delta g_n^m = w \delta t (m h_n^m + b u^2 + c u v + d v^2), \quad (2)$$

where b , c , and d are linear combinations of the coefficients g_n^m , h_n^m , with $m' = m - 2, m - 1, m, m + 1, m + 2$, but with all the terms having the same value of n . Thus while the secular variation produced by the drift is proportional to w , the first order terms in u and v have cancelled. This confirms the intuitive result that the effect of a small 'westward' drift is going to be very insensitive to a small change of drift axis. It follows that the values found for u and v will be much more uncertain than those for w . Intuitively, if w is uncertain by, say, 10%, then we would expect u and v to be uncertain by about 0.1 rad, about 6° .

For a given epoch, the problem of estimating the best values of the parameters u , v , w by least squares can now be considered, that is, the minimisation of the sum S of (1). This corresponds to fitting a 'dependent' variable by a function of an 'independent' variable, the function having known form but involving the parameters which are to be determined. The coefficients of the integrated secular variation, ΔG_n^m , ΔH_n^m , are the values of the 'dependent' variable and, although specified, they are subject to considerable error. They are to be fitted by δg_n^m , δh_n^m , which are complicated functions of the values g_n^m , h_n^m of the 'independent' variable (which in comparison are effectively exact), and of the parameters u , v , w , which are to be estimated. The resulting estimates of these parameters will be subject to errors, which will increase as the errors in ΔG_n^m , ΔH_n^m become larger. As the rotation of axes does not introduce any terms of different n , the separation of S into contributions of different n is still possible. If these contributions were separately minimised five different values of Ω would be obtained, which can be called Ω_n . (Richmond³ showed that, for drift about the geographic axis, the w_n ranged between 0.01 and 0.23° yr⁻¹.) Malin⁴ estimated the standard deviations of ΔG_n^m , ΔH_n^m used by himself and Saunders¹. The fractional errors were about 10% for $n = 2$, increasing to about 50% for $n = 6$. Clearly the Ω_n would be subject to considerable errors, larger with increasing n . (For drift about the geographical axis it can be shown, using conventional error theory, that Malin's figures would give standard deviations of w_n varying from 0.01 to 0.04° yr⁻¹; the effect of introducing the other two parameters u and v is probably small, but will always increase these values.)

Because of the cancellation in (2) of the first order terms in u and v , the least squares problem is non-linear even to the first approximation, and it is not possible to perform an analysis of its solution. The α_n parameters in (1), however, clearly behave as 'weights' in the least squares solution; if one of the α_n parameters were considerably larger than the others then Ω may be expected to depend mainly on that Ω_n .

In the discussion of their results, Malin and Saunders interpreted Ω as the angular motion of the outer layers of the electrically conducting fluid core. The magnetic lines of force in the core are probably 'frozen' to the fluid on the short time scales of the secular variation, and the interpretation of Malin and Saunders would be reasonable if the functions they had correlated were the radial magnetic fields at the surface of the core. It appears, however, that what they did correlate were the magnetic potentials at the surface of the Earth. For a unit amplitude harmonic n , m , the mean square potential on the Earth's surface is $1/(2n + 1)$, whereas the mean square radial field is $(n + 1)^2/(2n + 1)$, and the extrapolation down to the core gives another factor of $(1.83)^{2n}$; the α_n used by Malin and Saunders over-weighted the $n = 2$ harmonics by about 700:1 compared with the $n = 6$ harmonics. This would not have mattered if the field rotated bodily without changing its pattern, but different harmonics do have different drift rates, and the drift contributes only about half of the secular variation. Rich-

mond³ has shown the large differences in w which result from using these different weightings. Therefore the Ω obtained by Malin and Saunders are almost certainly not those appropriate to their interpretation.

Because of the very high weighting of the harmonics of low degree, the fairly smooth variation of Ω is probably mainly an indication of the fairly smooth, and comparatively well determined, changes of the small number of $n = 2$ coefficients. Their weighting also means that Ω will have smaller errors than the correct Ω ; for drift about the geographic axis, and using the standard deviations of Malin⁴, their weighting gives a standard deviation of w of about 0.005° yr⁻¹, whereas the correct weights give about 0.022° yr⁻¹, corresponding to about 1½° and 7° respectively for the axis directions. So a recalculation of Ω using the correct weights would be expected to give a much bigger scatter of the drift axes, and make any movement of the axes much more difficult to determine.

But using coefficients up to only $n = 6$ to calculate Ω does not in any case give a reasonable approximation to the rotation of the core surface. The contribution of the secular variation coefficients of a given degree, n , to the mean square field at the core surface forms one term of a series which shows no sign of convergence up to $n = 6$, and the contribution of the higher coefficients is not known⁵.

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DRS MALIN AND SAUNDERS REPLY: Lowes questions our conclusion on the grounds that the pole positions are not sufficiently well determined to show significant departures from the geographical pole.

In the absence of a satisfactory method for estimating the standard errors of the pole coordinates (Lowes gives an intuitive figure of about 6° , whereas our intuition, based on consistency of results, would give a smaller figure), we will attempt to test the validity of our results by the traditional method of a 'crucial experiment'. If our results are valid, they would predict that the optimum pole positions for epochs before 1940 would lie near the magnetic pole-Novaya Zemlya line and that their distance from the geographical pole would increase with the remoteness of the epoch. Further, we would expect the rotation rate to be smaller for earlier epochs. Alternatively, if the pole of rotation is really the geographical pole, we would expect the earlier optimum pole positions to be randomly distributed about the geographical pole.

We selected a uniform set of spherical harmonic models for the epochs 1905, 1915, 1925, 1935, 1945, derived from the main field and secular change models of Vestine *et al.*¹ and listed by Barraclough². The models are to the sixth degree and order, and are based on grid-point values of North and East magnetic intensity read from charted values of survey, repeat station, and observatory data. Although these data are less uniformly distributed with time than those used in our previous study, they are the best available at present. The method of determining the optimum pole positions and rotation rates is the same as that which we used previously³, and the results are given in Table 1. The results for 1925-1935 are in parenthesis because the rotation

TABLE 1 Pole positions and rotation rates required to give maximum correlation between magnetic field models for different epochs

Epochs	Mean epoch	Interval T (yr)	Pole position		Rotation δ/T (degrees yr ⁻¹)
			E. Longitude λ' (degrees)	Co-latitude, θ' (degrees)	
1905-15	1910	10	65.2	82.4	0.134
1905-25	1915	20	62.4	77.9	0.125
1915-25	1920	10	58.5	72.8	0.114
1905-35	1920	30	60.0	72.5	0.111
1915-35	1925	20	55.7	65.5	0.093
1905-45	1925	40	56.3	65.4	0.094
1915-45	1930	30	51.3	52.4	0.053
1925-35	1930	10	(52.4)	(41.8)	(0.007)
1925-45	1935	20	57.3	27.2	-0.077
1935-45	1940	10	58.6	23.6	-0.098

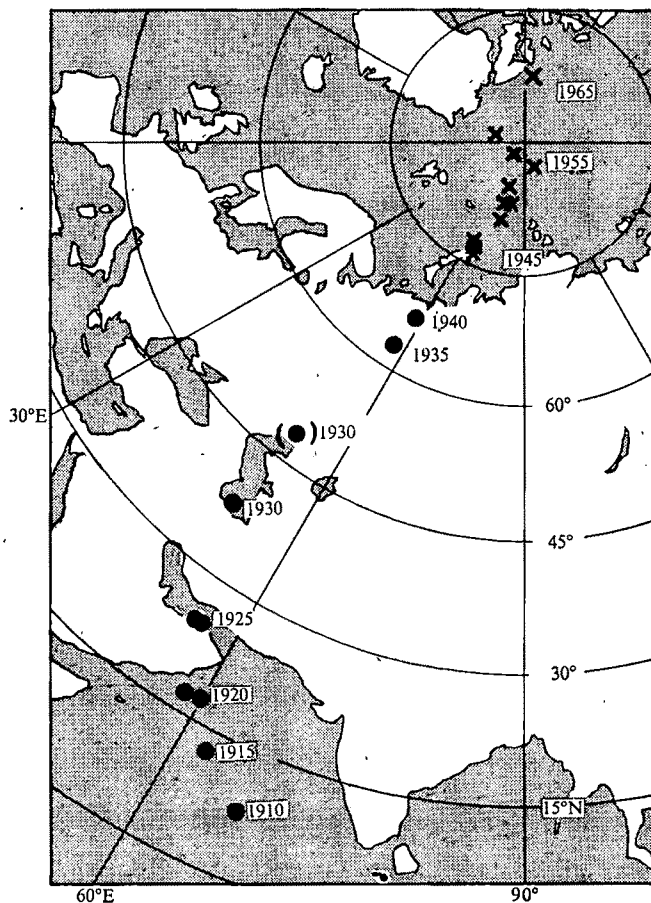


FIG. 1 Position of the optimum pole of rotation of the non-dipole part of the geomagnetic field.

between these epochs is very small and therefore the pole position is poorly determined (for a zero rotation the pole position would be indeterminate). In fact, the 1925-1935 correlation coefficient shows several maxima, and the position quoted represents only a local maximum.

The pole positions are plotted as circles on a zenithal equidistant chart (Fig. 1), together with the pole positions (indicated by crosses) obtained previously³. The results are in excellent accord with the prediction based on our earlier results. The second prediction, that the rotation rate should be smaller for earlier epochs, is also confirmed, though rather more spectacularly than we might have expected, because the rotation rate apparently drops to zero near 1930, and for earlier epochs shows the opposite sign. This is illustrated in Fig. 2, again circles and crosses denote present and previous results respectively.

Thus, the present results amply confirm our conclusion that, in general, the optimum pole of rotation of the non-dipole part of the geomagnetic field differs significantly from the geographical pole.

We have also suggested a physical interpretation of the phenomenon, based explicitly on the assumption that the rotation of the field reflected similar movements of the outer layers of the core. There are two separate parts to this assumption: (i) that the field rotates as a whole; (ii) that this rotation corresponds to a similar rotation of the outer core. It is the first of these parts that has been challenged by Lowes.

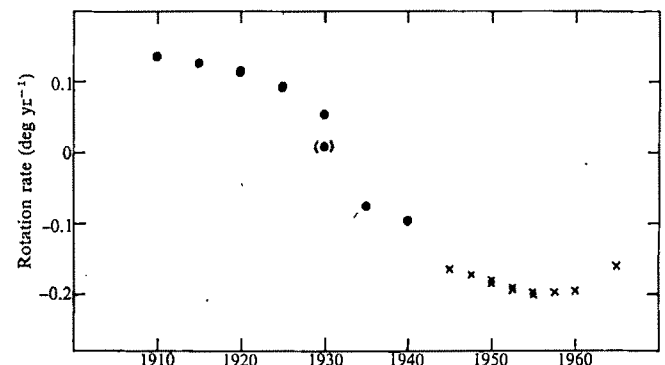


FIG. 2 Rate of rotation of the non-dipole part of the geomagnetic field about the optimum pole.

Lowes uses 'field' to denote the magnetic intensity, whereas, following Chapman and Bartels⁴, we allow it a more general meaning. Also, for 'rotation of the field', we should have written 'rotation of inhomogeneities of the field' (F. J. Müller, private communication), since rotation of an axi-symmetric field about its axis of symmetry would produce no secular change and therefore would not be detected in our investigation. For convenience, however, we retain the shorter notation.

If the whole of the secular change could be fitted by a simple rotation of the main field there would be no problem, since all the features of the field (potential, components of intensity, and so on) would yield the same pole position and rate of rotation for whatever height or depth they were analysed. It is clear, however, that such a simple model cannot account for all the complex changes in the field pattern. In the presence of such non-rotational components the derived pole position and rate of rotation will depend on the relative weights given to the individual spherical harmonic coefficients. [Two extreme examples are the potential at the surface, considered here, where the weighting is $(2n + 1)^{-1/2}$ (to convert from Schmidt semi-normalisation to full normalisation), and the vertical intensity at the core-

mantle interface, favoured by Lowes, where the weighting factor is $1.83^{n+2} \cdot (n+1) \cdot (2n+1)^{-1/2}$.]

Lowes asserts that the most appropriate rotation to investigate in connection with motions of the outer layers of the core is that of the vertical intensity at the core/mantle interface, but goes on to show that, because of the high weight given to the poorly determined high-degree coefficients relative to the better determined low-degree coefficients, the resulting pole positions would be of low significance. He further suggests that secular change coefficients of degree greater than six, which are too small to be determined significantly at the surface, might well contribute a major proportion of the secular change at the core/mantle interface. For these reasons, we prefer to consider the rotation of the field at the surface, where the observations are made, and where the secular change can be well represented by spherical harmonic coefficients to the sixth degree and order. Assuming, as before, that the non-dipole field is 'frozen in' to the core, the argument may be stated as follows: if the core rotates about the deduced pole position at the deduced rate then it will reproduce the observed secular change as accurately as is possible with a simple rotation.

We concede that such a simple model is inadequate to account for all of the observed changes in the non-dipole field, but it represents a better approximation (even when the additional degrees of freedom are taken into account) than westward drift. Moreover, it has the merit of making predictions about the diurnal variation of latitude which may be tested by astronomical observation³. There seems little point in pursuing a more sophisticated physical model until the quality and distribution of secular change data permits a more reliable determination of high degree spherical harmonic coefficients.

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Electron diffraction on liquid water

X-RAY diffraction has been successfully used in determining radial distribution function in liquid water (see references in ref. 1). The few attempts²⁻⁷ to apply diffraction of a high energy electron beam to the same end in liquids in general has not led to usable diffraction patterns, particularly in the case of liquids of higher vapour pressure⁷. To overcome the difficulties arising from the strong absorption of electrons, we produced in an electron microscope a very thin layer of liquid water nearly in equilibrium with its vapour and prevented the pressure in the vicinity of the electron beam for increasing by evaporation. The liquid layer scattered the penetrating electron beam and usable diffraction patterns could be obtained.

A chamber⁸ for an electronoptical device Zeiss EF-4 was constructed for producing a water film of controlled thickness

and temperature. The film was created by wiping a drop of the liquid over a hole 2 mm in diameter in a copper plate of thickness 0.02. This was enclosed in a chamber connected to the vacuum space only by two apertures, one of diameter 70 μm for the entering primary beam and the other of diameter 200 μm for the scattered beam. Drop and film could be reproduced many times by a suitable mechanism.

In order to avoid quick disappearance of the water film, the vapour escaping through the apertures could be replaced by evaporating the water stored in the chamber.

Usually, the primary electron beam was completely absorbed immediately after the water film was produced. By slow evaporation the thickness of the film decreased and a pattern of diffuse Debye-Scherrer rings could be seen. This was photographed on a 9×12 cm Agfa-Gevaert Scientia 23D50 plate at distances of 20, 42 and 80 cm from the water film. Thus, the ranges of the scattering angle θ and the usual parameter $s = (4\pi/\lambda) \sin(\theta/2)$ could be varied. In order to avoid a predominance of scattering at very small angles, we used an s^2 rotating sector 2 mm over the photoplate. The intensity distribution $I(s)$ on the photoplate was determined by a Zeiss GIII Schnellfotometer. The exposure time was 1 to 3 s depending on the sample-to-plate distance. Short exposure times are of particular importance in scattering from solutions in which concentration changes during the experiment are to be avoided.

Figure 1 shows a diffraction photograph (a negative print) on water at 4° C. It was taken at a sample-to-photoplate distance of 42 cm using a monochromatic electron beam of energy ~ 67 keV, corresponding to a wavelength of 0.045 Å determined experimentally. The radial distribution function in the liquid was calculated by Fourier transformation of that part of the scattered intensity which could be assumed to be coherent scattering. This was determined arbitrarily in the

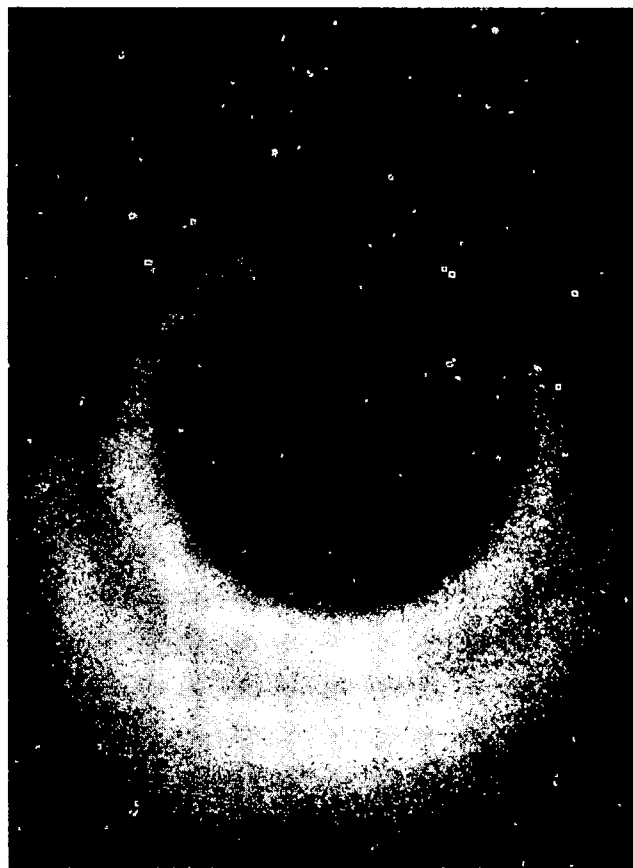


Fig. 1 Diffraction photograph (negative print) of water at 4° C.

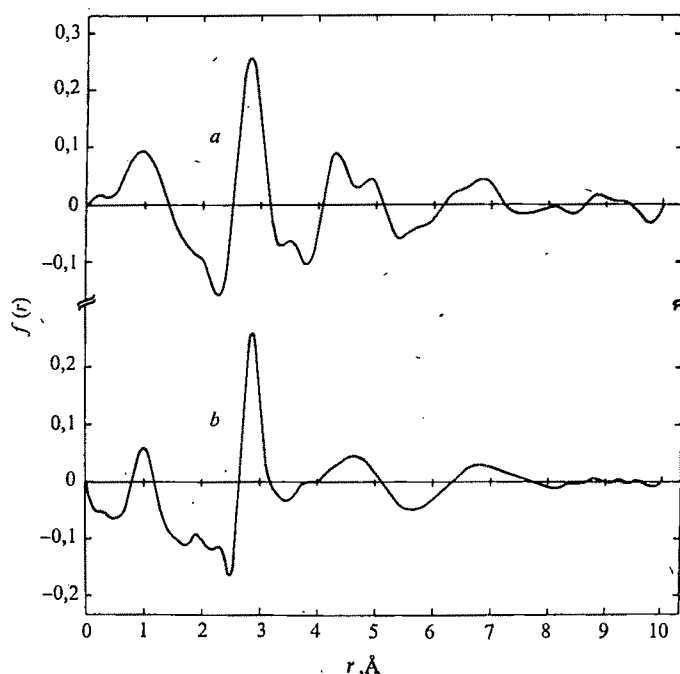


Fig. 2 Radial distribution function for liquid water, H_2O , at 4°C . a, Electron diffraction; b, X-ray diffraction^{1,9}.

following way. The modified experimental molecular intensities

$$sM(s) = s[I(s) - I_B(s)]/I_B(s)$$

were obtained by arbitrarily drawing in smooth background curves $I_B(s)$ on the total experimental molecular intensity $I(s)$ curves. The Fourier transformation

$$f(r) = \int_{s_{\min}}^{s_{\max}} sM(s) \sin(sr) ds$$

of the $sM(s)$ function was carried out in the s range 1.7 to 9.5 \AA^{-1} using $\Delta s = 0.10 \text{ \AA}^{-1}$ steps.

The obtained radial distribution curve (Fig. 2a), which is not much altered by variations in the background curve, shows almost exactly the same maxima as the corresponding curves obtained from X-ray diffraction (Fig. 2b)^{1,9}.

According to the interpretation of other authors, these peaks must be ascribed, in the order of increasing r , to interactions O—H (intermolecular), O...O interactions between immediate neighbour molecules and second and more distant neighbours.

In the interaction between radiation and matter, the main difference in principle between X rays and the electron beam is that the former are diffracted by the electron clouds whereas the latter is scattered by the electric potential, depending on the steric configuration of the nuclei and the density distribution of the electrons. Consequently, electron diffraction allows the determination of the position of light atoms in the presence of heavy atoms. Therefore, in the case of water, more reliable information about the hydrogen-bonds can be expected.

The energy of the electron beam generally used in diffraction experiments corresponds to a wavelength λ of some hundredths of an Angström. This is at least ten times less than the wavelength of the X rays used for structural studies. Thus, the s range used in Fourier transformation for obtaining radial distribution from coherent intensity is much greater for electrons and so the cutoff error is reduced by comparison with X rays.

The ratio of the intensity of coherently scattered radiation to that of incident radiation is a few orders of magnitude higher in the case of electrons than in the case of X rays.

This leads to the practical conclusion that although an exposure time of a few seconds is sufficient for electrons, several hours are necessary for X rays.

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BIOLOGICAL SCIENCES

Method for relating the structure and properties of chemical compounds

THE structure diagrams of chemical compounds are widely used in the communication of chemistry. They are also used in chemical information systems, mainly as keys for the retrieval of associated information¹. However some chemical information systems hold property data as well as structure diagrams in machine-readable forms²⁻⁵. If structure-property relationships could be investigated easily within chemical information systems, then the usefulness of the systems would be considerably increased. Described below are some results which have been obtained during investigation of structure-property relationships using a combination of regression analysis and some techniques of chemical structure handling used in information systems.

The method used is empirical⁶⁻⁸. The structures were coded manually as redundant connection tables and put in to the computer with the properties of the compounds. The rest of the processing was carried out automatically. A structural fragment was generated on each atom in each structure. Each fragment consisted of the central atom, the bonds it forms and the atoms to which it is bonded (excluding hydrogen atoms). Fragments of this type have been investigated⁹⁻¹⁰ and used as screens (M. F. Lynch *et al.*, personal communication, and ref. 11) in substructure search systems and for the automatic classification of chemical structures¹².

It was assumed that the value of the property under investigation, y , of the i th compound is related to its structure by the equation

$$y_i = \sum_{j=1}^n b_j x_{ij}$$

where there are n types of structural fragment in the set of structures and x_{ij} is the number of times that the j th fragment occurs in the i th structure. The constants b_j were determined by carrying out a regression analysis using a computer manufacturer's statistical analysis programs¹³.

The method was tested with a group of 79 penicillins and using the percentage serum binding as the property parameter¹⁴. They were chosen because they form a large group of structures for which property data have been published.

These compounds have also been studied using a semi-empirical structure-property correlation method and the results are available for comparison¹⁴.

An automatic analysis of the 79 penicillins in terms of the structural fragments described above showed that they contain 52 different fragment types. Eight of these however were derived from the penicillin nucleus and always occurred the same number of times in a structure. They were therefore omitted from the analysis. Also one group of four fragments and three groups of two fragments had within group correlation coefficients of 1 and thus only one fragment from each group was included. The regression analysis also excluded variables which were not significant at the 10% level.

The results of the regression analysis¹³ are shown in Table 1. The multiple correlation coefficient is 0.993 with 66 degrees of freedom ($F = 390$, $F_{12, 60, 0.001} = 3.32$) and the residual error 9.73. A regression carried out using log (amount of penicillin bound to serum/amount free) as the independent variable¹⁴ gave a multiple correlation coefficient of 0.945 with 70 degrees of freedom and a residual error of 0.294. It will be seen in Table 1 that all of the fragments except HO-C have regression coefficients which are different from zero at a significance level of 5%. The fragments which have negative regression coefficients and thus reduce serum binding are hydrophilic whereas the fragments with positive regression coefficients, which increase serum binding, are hydrophobic. The relationship between the hydrophobic nature of the side chain and serum binding of penicillins has been noted by other authors^{14, 15}.

This method differs from others published in that both the side chain and nucleus are treated in the same manner and both are broken down into fragments automatically¹⁶. It would be possible to integrate this kind of analysis easily into a computerised chemical information system. As structural fragments are developed from structure diagrams, the procedure will not resolve some isomers. This situation may be improved by using larger structural fragments, or more pre-

cise forms of structural representation than the connection table used in this work. The method is subject to the usual restrictions connected with the use of multiple regression analysis. We hope to publish a more detailed account of this and related work in the near future.

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TABLE 1 Regression analysis of structural fragments

Fragment type	Regression coefficient	Student <i>t</i> statistic (66 d.f.)	Perfectly correlated fragments
NH ₂ - S	-30.64	2.23	$\begin{array}{c} \text{O} = \text{S} \\ \\ \text{NH}_2 - \text{S} - \text{C} \\ \\ \text{O} \end{array}$
Br - C	27.50	4.55	
C * S * C	17.72	3.57	
F - C	15.81	4.32	$\begin{array}{c} \text{S} \\ \\ \text{C}^* \text{C}^* \text{C} \end{array}$
NH ₂ - C	-19.69	6.18	
Cl - C	20.21	13.04	
C - CH ₂ - C	10.88	8.53	$\begin{array}{c} \text{C} \\ \\ \text{S}^* \text{C}^* \text{C}^* \\ \\ \text{F} \\ \\ \text{C}^* \text{C}^* \text{C}^* \end{array}$
C - O - C	6.97	2.76	
HO - C	-11.65	1.89	
CH ₃ - C	8.17	3.92	$\text{C} - \text{NH} - \text{C}$
O = C	-27.47	2.68	
C * CH * C	9.64	14.77	
Regression constant	91.04	2.74	

Results of the regression analysis using the serum binding percentage as the dependent variable and the occurrence of structural fragments as independent variables. The perfectly correlated fragments have correlation coefficients of +1 with the fragments in the left hand column and were not included in the calculation. All bonds are in chains except those denoted by asterisks which are delocalised ring bonds.

Terminal riboadenylate transferase in human lymphocytes

TERMINAL riboadenylate transferase (TrT) catalyses the transfer of adenylate residues from ATP to the 3'-hydroxyl group of certain polyribonucleotides in the presence of Mn²⁺. Enzymes of this kind may be involved in the processing of heterogeneous nuclear RNA to mRNA in eukaryotic cells, since many types of mRNA have terminal poly(A) sequences. Evidence for this is rather fragmentary and is complicated by reports of several forms of poly(A)-polymerising activities in both nucleus and cytoplasm¹⁻¹⁰. Clearer evidence might be obtained from systems that could be experimentally manipulated to show variation in the levels of activity of poly(A) polymerases and rates of mRNA synthesis. We have found that human lymphocytes exhibit increased TrT activity as part of their response to stimulation by phytohaemagglutinin (PHA) *in vitro*. This is the first mammalian, non-viral system in which increases in TrT activity have been observed with changes in the physiological state of the cell. The PHA-stimulated lymphocyte may be suitable for combined polymerase-mRNA turnover studies designed to clarify enzymatic steps in mRNA processing and terminal poly(A)

TABLE 1 Stimulation of polymerase activities in lymphocytes cultured with phytohaemagglutinin

Lymphocytes	Terminal riboadenylate transferase		Nucleus		Maxipolymerase		Minipolymerase	
	Bulk	Gradient	Bulk	Gradient	Cytoplasm Bulk	Nucleus Bulk		
Unstimulated (48 h)	28	24	0	0	1.3		0.14	
	33	15	0	0	1.2			
	21	21	0	0	1.3		0.14	
PHA-stimulated (48 h)	81	80	13	0	7.8		0.76	
	84	94	9	0	8.1		0.24	
	—	127	0	0	5.6		0.54	
	69	60	—	—	5.7		0.23	
	119	—	6	6	7.7		0.40	

Lymphocytes were cultured in RPMI-1640 tissue culture medium (Gibco) supplemented with 20% foetal calf serum, penicillin and streptomycin. Stimulated cultures (a) contained 0.2 ml Difco PHA-P stock PHA per 250 ml and controls (b) received no PHA. After the appropriate time, lymphocytes were suspended to a cell density of 1×10^6 cells ml^{-1} in TKM-sucrose buffer containing 50 mM Tris-HCl pH 7.6, 25 mM KCl, 5 mM MgCl_2 , and 0.25 M sucrose. The cells were counted, homogenised and fractionated by differential centrifugation¹⁷. The crude fractions were assayed for several enzymes directly and after velocity sedimentation through a 5–20% sucrose density gradient¹⁷. Assays for maxi and mini DNA-dependent DNA polymerases were as previously described¹⁷. The bulk assays have a total volume of 250 μl and the gradient assays were in 60 μl . The assays for TrT activity contained 0.2 M Tris-HCl, pH 8.25, 0.01 M rA(pA)₁₈, 0.5 mM ^3H -rATP (3.9 c.p.m. pmol^{-1}), 0.5 mM MnCl_2 , 4mM 2-mercaptoethanol, 0.1 mg ml^{-1} bovine serum albumin and enzyme. Reaction mixtures were incubated at 35° C. For the bulk assay aliquots were removed from the reaction mixture at various times, spotted on GF/C filters, and processed for acid-insoluble activity¹⁷. Enzyme-specific activity is expressed as nmol nucleotide incorporated per 10^6 cells h^{-1} . Each line in the table represents a separate experiment.

addition, since Rosenfeld *et al.*¹¹ have reported increases in poly(A)-rich mRNA during lymphocyte transformation by PHA.

For our studies, normal human lymphocytes were separated on Hypaque-Ficoll gradients¹² and incubated with PHA (Table 1). Stimulation was monitored morphologically and there was routinely 60% blast transformation after 48 h. Thymidine pulses indicated maximal DNA synthesis at 72 h and the number of cells had approximately doubled by 96 h. For enzyme analysis cultured lymphocytes were collected by centrifugation, washed in TKM sucrose, disrupted by homogenisation, and separated into nuclear and soluble cytoplasmic fractions by differential centrifugation. In some experiments, nuclear and cytoplasmic extracts were layered on sucrose density gradients for velocity gradient fractionation. TrT and several DNA polymerase activities were assayed (Table 1.) The oligonucleotide normally used to assay TrT, rA(pA)₁₈, was prepared by controlled hydrolysis of poly(A)₁₃.

Increases in polymerase activities following treatment of lymphocytes with PHA are shown in Table 1. A typical velocity gradient separation of polymerase activities from 48 h stimulated lymphocytes is presented in Fig. 1. Direct assay of nuclear and cytoplasmic extracts (bulk assays) and

summation of gradient activities showed that most of the total TrT activity was recovered from the gradient in a single peak with an estimated molecular weight of 60,000. The stimulation of the cytoplasmic high molecular weight or 'maxi' DNA polymerase confirms the work of other investigators and will not be discussed further^{14,15}. The activity of the low molecular weight or 'mini' DNA polymerase found in the nucleus may also increase, but its activity was always much lower than that of the cytoplasmic DNA polymerase (Table 1).

The time courses of TrT, maxipolymerase and thymidine incorporation during PHA stimulation are illustrated in Fig. 2. In general TrT activity increases three to six-fold, reaching a maximum after about 48 h. Since the cytoplasmic TrT

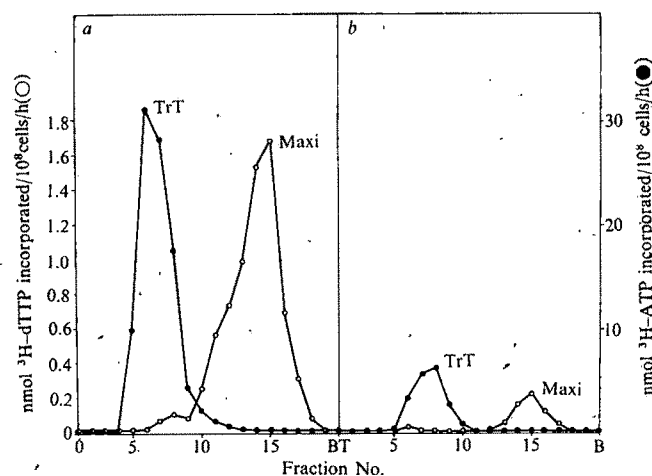


FIG. 1 Velocity gradient fractionation of polymerase activities. The soluble, cytoplasmic supernatant fractions (0.25 ml) prepared as described¹⁷ were layered on 5–20% sucrose density gradients in 50 mM Tris-HCl pH 7.6, 100 mM KCl, 1 mM 2-mercaptoethanol and 0.1 mg ml^{-1} bovine serum albumin. The gradients were centrifuged for 16 h at 40,000 r.p.m. (100,000g) and then collected in 20 fractions. Enzymes were assayed as described in Table 1. a, Stimulated lymphocytes; b, normal lymphocytes.

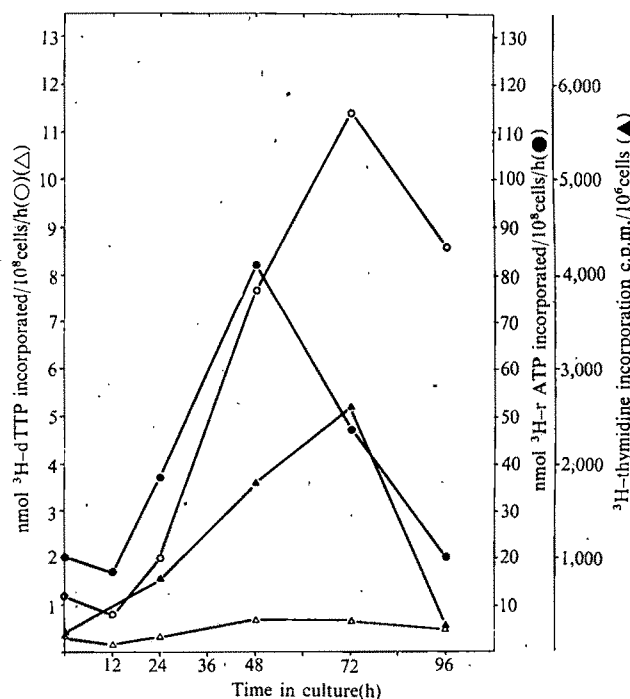


FIG. 2 Variation of polymerase activities with time after addition of PHA to cultures of human lymphocytes. For thymidine pulses, 1×10^6 cells were incubated for 2 h with 5 μCi ^3H -thymidine at 37° C. The cells were then pelleted and precipitated with cold 5% trichloroacetic acid (TCA). The TCA precipitate was collected on GF/C disks, washed, dried and counted in a liquid scintillation counter. Thymidine incorporation was linear during this time. Enzyme assays are described in Table 1. ●, Cytoplasmic TrT; ○, cytoplasmic 'maxi'; ▲, ^3H -thymidine incorporation; △, nuclear 'mini'.

assay was linear with both time and concentration of extract when a long oligoadenylate initiator was used, we feel this result is unambiguous. Cytoplasmic DNA polymerase activity and thymidine incorporation peaked at 72 h just before cell division. Most cells underwent mitosis between 72 and 96 h with a doubling in cell number. Synchronisation of cell division is good in this system. Increase in TrT activity in lymphocytes was prevented by cycloheximide addition at any time during lymphocyte activation by PHA.

The TrT activity which we detected was a soluble enzyme in the cytoplasmic fraction, was Mn^{2+} -dependent, required an oligonucleotide initiator and had a molecular weight of approximately 60,000. In crude extracts we found that an initiator 12–16 nucleotides long was optimal for detecting this activity. A low level of activity was found in the nucleus, but this result is difficult to interpret, since the enzyme could have leaked from the nucleus or bound adventitiously to chromatin upon cell breakage.

Table 2 illustrates the effects of several chemical compounds on TrT activity. Rifampicin-SV, α -amanitin and actinomycin D are all inhibitors of mammalian DNA-dependent RNA polymerases. These drugs have no effect

TABLE 2 Effect of chemicals on terminal riboadenylate transferase activity

Compound added	Percent inhibition
α -Amanitin	
8.5×10^{-6} M	1.2
1.4×10^{-6} M	0
Rifampicin	
96 μ g ml ⁻¹	0
160 μ g ml ⁻¹	0
Proflavin sulfate	
10 μ g ml ⁻¹	17
100 μ g ml ⁻¹	98
Actinomycin D	
20 μ g ml ⁻¹	3
50 μ g ml ⁻¹	2
N-Ethylmaleimide (NEM)	
0.8 mM	82
4 mM	99

TrT assays were carried out as described in Table 1. All compounds except NEM were added to the reaction mixtures. For experiments using NEM, the supernatant fraction was incubated in ice for 10 min with NEM before the addition of reaction mixture.

on TrT activity at concentrations which would inhibit RNA polymerase. Proflavin sulphate at a concentration of 100 μ g ml⁻¹ inhibits TrT. This compound also inhibits the purified TrT from calf thymus (F.J.B. unpublished data) and a similar activity in vaccinia cores¹⁶. Human TrT is a sulphhydryl enzyme, since it is completely inhibited by 4 mM N-ethylmaleimide (NEM).

These findings indicate that TrT is an enzyme distinct from DNA-dependent RNA polymerases. Its activity increases three to six-fold during PHA stimulation of the human lymphocyte. The role it plays in RNA processing and the addition of poly(A) sequences to the 3'-terminus of mRNAs may be studied more rigorously in this system which exhibits biological variation.

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Is terminal deoxynucleotidyl transferase a somatic mutagen in lymphocytes?

AN enzyme able to add deoxyribonucleotides to the ends of DNA primers has been identified in calf thymus by Bollum and his colleagues and named terminal deoxynucleotidyl transferase^{1–4}. Chang⁵ has indicated that the enzyme is a specialised constituent of thymus cells and is not present in any other organ of several animal species. The enzyme is located in the lymphocyte fraction of the thymus (Silverstone, Parkman, McCaffrey and Baltimore, unpublished results). Recently, we found an enzyme with the properties of a terminal transferase in circulating lymphoblasts of patients with acute lymphoblastic leukaemia (ref. 6 and McCaffrey, Harrison, and Baltimore, unpublished results).

It seemed to me possible that terminal transferase could act as a mutagen, diversifying those molecules of the T lymphocyte which carry the immunological specificity of the cell. Although I realise that the question of which molecules give specificity to thymus-derived lymphocytes is open^{7,8}, I have assumed that the molecules are probably similar, if not identical, to antibody molecules. The hypothesis I wish to discuss is therefore that terminal transferase acts as a somatic mutagen to diversify the amino acid sequence in the variable region of immunoglobulin molecules. There are various hypotheses as to how the variety of amino acid sequences arises in the V regions of immunoglobulins⁹. I have assumed that somatic mutation plays a significant role: if all antibody synthesis is directly specified by inherited V regions^{9,10}, my proposal is clearly meaningless.

Numerous models of immunoglobulin diversification^{11–17}, which originate in the writing of Lederberg¹⁸ and Burnet¹⁹ postulate that most V regions arise by a process of somatic mutation onto which is superimposed a selective pressure. Starting from these models, I wish to suggest, as did Brenner and Milstein²¹, that the mutations arise because single-stranded gaps are made in an inherited gene which encodes the V regions of an immunoglobulin, and that during the repair of these gaps, an enzyme inserts the wrong base (or wrong bases), causing a mutation. Terminal transferase

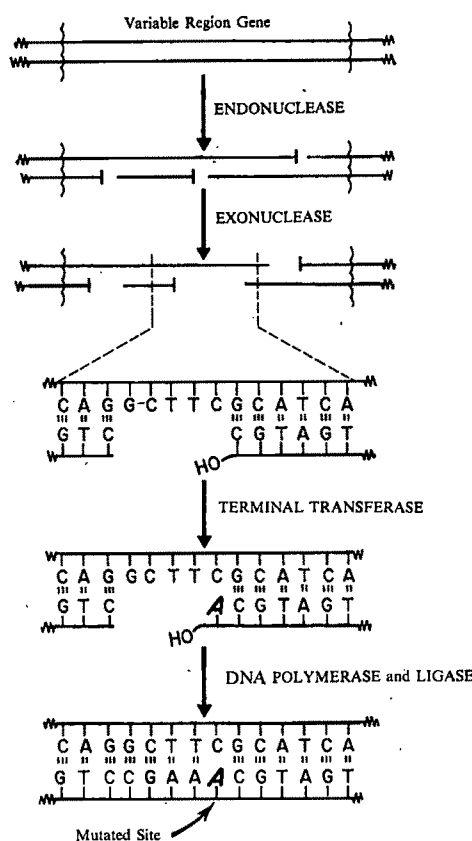


Fig. 1 Schematic representation of hypothetical mutagenic activity of terminal transferase in concert with other enzymes.

would be the mutator and thus would be the somatic 'generator of diversity'¹².

The biochemistry of terminal transferase is consistent with this model. The enzyme will add mononucleotides sequentially to any 3'-OH-terminated segment of DNA and will add the nucleotides independent of any template direction³. Ordinarily, free single strands are used as primers but the enzyme can act at gaps in double-stranded molecules almost as effectively as it does on single-stranded primers (ref. 20 and Panet, McCaffrey, Smoler and Baltimore, unpublished results).

Because terminal transferase acts non-specifically at 3'-OH ends, it must be directed to the appropriate V region in order to act on that gene but not on others. One possibility is that immune lymphocyte precursors contain a site-specific endonuclease which produces, possibly in concert with an exonuclease, the gaps at which terminal transferase can act.

A specific form of this model is presented schematically in Fig. 1. I postulate that an endonuclease makes several single-strand scissions in the V region gene of an immunoglobulin chain. Three sites of endonuclease cleavage in the V region gene are suggested because of the data from Wu and Kabat²¹ showing three hot spots of variability in the V region (hypervariable regions). The nicks are then elongated to variable length gaps by an exonuclease. The repair of one gap is shown in detail (Fig. 1). Initially, terminal transferase inserts a single, random nucleotide which, in this case, does not form a base pair with the nucleotide on the opposite strand and is therefore a mutated site. The gap is then filled by an ordinary DNA polymerase and sealed by a ligase. The filling of a gap containing a mismatched base pair at the 3'-OH end can be catalysed by a mammalian DNA polymerase²².

I postulate that a process such as that depicted in Fig. 1 causes mutations at the hypervariable positions in the V region genes^{21,23}. There are extensive differences in amino

acid sequence between V regions which are located outside the hypervariable regions. Such variation is probably due to multiple, inherited genes and not to somatic mutation²⁴⁻²⁶. These inherited differences include differences between species, differences between the subclasses of variable regions within the antibodies of a given species and differences due to allelic genetic polymorphisms in a single gene. It is significant that when data on one class of antibodies in a single inbred strain—the lambda chains of Balb/c mice—were collected^{27,28}, all the amino acid substitutions were in the hypervariable regions. In kappa chains of Balb/c mice, however, extensive variation occurs throughout the V region, suggesting the existence of many inherited V region genes²⁴. Even in the kappa chains, however, when proteins were analysed which were virtually identical outside the hypervariable regions, variability localised to the hypervariable regions was evident²⁶.

All this speculation relates to the production of circulating antibodies, but terminal transferase is found in thymocytes and it is not clear whether such cells have antibody on their surface. Some class of molecules resembling antibody must be involved in T cell specificity, however, and whatever those molecules, it seems reasonable that some of their variability arises in a manner similar to that of antibody variability.

To try to relate this model to antibody synthesis by B lymphocytes and plasma cells, we have sought terminal transferase in cells from the chicken's bursa of Fabricius. In agreement with previous results⁵, no terminal transferase like that in the thymus was evident in bursal cells (McCaffrey, Smoler and Baltimore, unpublished results). A high concentration of a unique replicative DNA polymerase which might make mistakes is, however, found in bursal cells (McCaffrey, Smoler and Baltimore, unpublished results). Immunoglobulin synthesis in B cells might therefore also involve a process of somatic diversification analogous to that described here for thymus cells. There could, however, be significant differences between the mechanisms of T and B cell diversification.

The molecular model suggested here is much more detailed than is justified by existing data. It is presented merely to show the context in which terminal transferase might act as a mutagen to diversify immunoglobulin structure. The only elements I wish to stress are the potential ability of the enzyme or enzymes like it to produce somatic variability in an inherited gene and the occurrence of such an enzyme in thymic lymphocytes. Terminal transferase could, for example, be involved in the type of somatic diversification discussed by Cunningham²⁹.

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Complementation of a defect in the production of ribosomal RNA in somatic cell hybrids

THE use of cell hybridisation for complementation tests is hampered because the establishment of a hybrid cell line may involve rearrangement of both parental cells' genetic material, and often loss of chromosomes¹⁻³. Since it is difficult to recognise single chromosomes, the genotype of the hybrid cannot be determined easily. Furthermore, strong selective pressures are often applied to isolate hybrid cells. Thus, the appearance of the wild type phenotype in a hybrid between a mutant and a wild type cell does not always imply that the mutation is recessive and can be complemented by the wild type allele: alternative hypotheses must be considered.

We isolated⁴ a temperature sensitive (*ts*) mutant from the Syrian hamster cell line BHK21/13, *ts422E*, which cannot grow at 39° C because at this temperature a block after formation of the 32S precursor prevents the production of 28S rRNA and 60S ribosomal subunits in the cytoplasm, while 18S rRNA and 40S ribosomal subunits are produced normally. The mutation seems to be in a gene coding for a protein involved in the processing of rRNA, the most likely possibilities being a ribosomal protein or an enzyme⁴.

The mutation seems to be recessive as it can be complemented in intraspecific hybrids with many other *ts*BHK mutants. These hybrids can all grow at 39° C⁵. In principle, the *ts422E* defect could be complemented in a hybrid cell by two different mechanisms. (a) The processing defect of *ts422E* could be relieved by the gene product of the wild type allele so that 28S rRNA transcribed from both parental cell genomes would be produced at 39° C. (b) The wild type phenotype could also be produced if synthesis of rRNA in the hybrid cells were sustained exclusively by the *ts** parental genome, while production of rRNA transcribed from the *422E* genome would still be inhibited. The latter mechanism would imply that the *ts422E* mutation is not recessive, but rather partially dominant, or else it is a mutation which cannot be complemented in *trans*. We have therefore investigated how the *ts422E* mutation is complemented in hybrid cells. We took advantage of the differences in the electrophoretic mobility of the 28S rRNAs of different species^{6,7}. Since it has been shown that in mouse-hamster hybrids, the 28S rRNA of both species is synthesised⁸, we isolated hybrids between *ts422E* and mouse cells, and determined the type of 28S rRNA formed at 33° C and 39° C.

Cell culture conditions were as described before^{4,5}. *ts422E* cells were hybridised with two lines of mouse cells, C2F, a thymidine kinase deficient derivative of 3T3 cells⁹, and TG8, a hypoxanthine, guanine phosphoribosyl transferase (HGPRT) deficient derivative of 3T6 cells¹⁰. Cells were fused in the presence of β -propiolactone inactivated Sendai virus, and incubated at 33° C for 2 d. Hybrids were selected at 39° C in HAT (aminopterin 10⁻⁵ M, thymidine 4 \times 10⁻⁵ M, hypoxanthine 10⁻⁴ M) medium¹¹. *ts422E* cannot grow at 39° C and the mouse parental cells are killed by the HAT medium. Hybrid colonies appeared with a frequency of about 10⁻⁴. Colonies were isolated and propagated at 39° C in HAT medium for two passages before transfer to 37° C in non-selective medium. Their chromosomal constitution was determined as described¹². 28S rRNA was analysed in six hybrid clones, four from the C2F cross and two from the TG8 cross, selected on the basis of their chromosome complement, that varied from a majority of hamster chromosomes

TABLE 1 Production of hamster and mouse 28S rRNA in hybrids between *ts422E* BHK and mouse cells

Cells	Modal chromosome No.		Total production of 28S rRNA†	39° C		Total production of 28S rRNA†	33° C	
	+ acrocentric	Bi-armed		% Mouse	% Hamster‡		% Mouse	% Hamster‡
Parents								
<i>ts422E</i> (BHK)	9	30	<0.05	—	—	1	0	100
C2F (3T3)	68	0	1	100	0	1	100	0
TG8 (3T6)	90	0	1	100	0	1	100	0
Hybrids								
C12	70	58	1	15	85	1	10	90
C16	63	27	1	50	50	1	51	49
T10*	54	29	1	80	20	1	81	19
T2	82	48	1	41	59	1	43	57
C1	63	33	1	35	65	1	33	67
C7	38	56	1	26	74	1	29	71

* This hybrid had a bimodal chromosomes distribution, with about one-third of the mitotic figures having a modal number of 85 telocentric and 43 bi-armed.

† In arbitrary units, 1 corresponds to the ratio radioactivity in 28S rRNA/radioactivity in 18S rRNA obtained in wild type cells labelled with ³H-uridine for 2 h at 39° C or 3 h at 33° C, as described in Fig. 1.

‡ Calculated as described in Fig. 1.

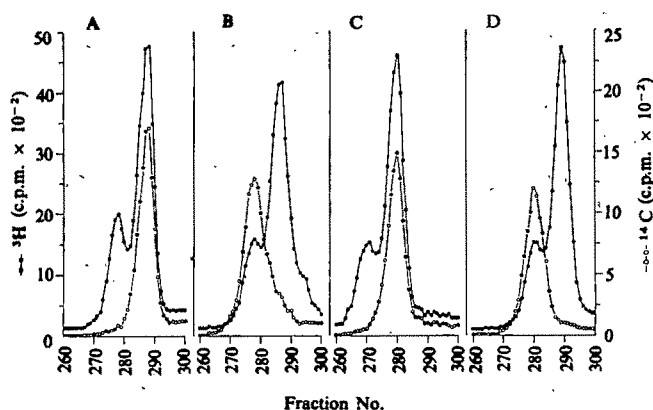


Fig. 1 Polyacrylamide gel electrophoresis of 28S rRNA from hybrid C7. Cells were shifted to 39° C or 33° C and after 2 d pulsed with ^3H -uridine ($4 \mu\text{Ci}$, $0.04 \mu\text{g ml}^{-1}$) for 2 h at 39° C and 3 h at 33° C. Cells were then washed twice with cold, isotonic Tris-buffered saline (pH 7.4) and the cytoplasmic fraction was prepared as previously described⁴. RNA was purified by two phenol extractions at room temperature and precipitated with two volumes of 95% ethanol, at -20° C. RNA was layered on linear 15-30% sucrose gradients (34 ml) in SDS buffer (0.1 M NaCl-0.5% SDS-0.01 M Tris-HCl, pH 7.2) and centrifuged at 24,000 r.p.m. for 16 h at 23° C in a SW 27 Spinco rotor. For each sucrose gradient, 1 ml fractions were collected from the bottom of the tube and the radioactivity of an aliquot was counted. The 28S rRNA peak was pooled and ethanol precipitated. 28S rRNA was fractionated by polyacrylamide gel electrophoresis: gels contained 2.6% (w/v) acrylamide and 0.13% (w/v) N, N'-methylene bisacrylamide, and were polymerised in a buffer containing 20 mM Na acetate, 1.0 mM NaEDTA, 40 mM Tris-acetate, pH 7.2, 0.2% SDS, 10% glycerol. Electrophoresis was run at room temperature, at 5 mA per gel, for 48 h in a buffer containing Tris, pH 7.2, Na acetate, NaEDTA and SDS in the same concentrations as in the gel. The desired portion of the gel was sliced frozen into 1 mm slices, solubilised in 0.5 ml of an NCS-water mixture (9:1) at 60° C overnight, and counted in a toluene-based scintillation fluid. Each 28S rRNA sample was run together with a ^{14}C -labelled marker of 28S rRNA prepared from 3T3 mouse cells or BHK cells. A, 28S rRNA (^3H) from hybrid C7 growing at 33° C (●), hamster 28S ^{14}C rRNA (○); B, 28S rRNA (^3H) from hybrid C7 growing at 39° C (●), mouse 28S ^{14}C -rRNA (○); C, 28S rRNA (^3H) from hybrid C7 growing at 39° C (●), hamster 28S ^{14}C rRNA (○); D, 28S rRNA (^3H) from hybrid C7 growing at 39° C (●), mouse 28S ^{14}C rRNA (○). Electrophoresis is from left to right. The percentage of mouse and hamster 28S rRNA produced by the hybrid was estimated by calculating the areas under each curve, which were assumed to be symmetrical.

to a majority of mouse chromosomes. Since the mouse parental lines are free of bi-armed chromosomes, the chromosome contribution of each species to the hybrids can be determined by assuming that the ratio of bi-armed to telocentric of the parental hamster cells remains unchanged (Table 2).

For the preparation of 28S rRNA, cells were labelled with ^3H -uridine for 2 h at 39° C and 3 h at 33° C (Fig. 1), to ensure that the appearance of 28S rRNA in the hybrids at

39° C would not be due to the small amount of 28S rRNA produced in *ts422E* cells at this temperature; this is generally not detectable with pulses of up to 2 h⁴. A normal amount of 28S rRNA was produced in all hybrids at both temperatures.

On polyacrylamide gels hamster 28S rRNA moves ahead of mouse 28S rRNA⁸. The 28S rRNA from each gradient was analysed on 2.6% polyacrylamide gels, 35 cm long, run for 48 h, together with appropriate markers. The separation obtainable in these conditions is shown in Fig. 1.

A summary of the results obtained with all hybrid clones examined is shown in Table 1. Hamster 28S rRNA was synthesised in all hybrids at both temperatures. The amount of hamster 28S rRNA in the hybrids varies from 20% to 90% of the total 28S rRNA, but in each hybrid was the same at 33° C and 39° C. Thus, the mutation of *ts422E* cells can be complemented by the wild type allele provided by the mouse genome.

To show that this was not due to extensive chromosomal rearrangements or selection of hybrid lines with specific chromosome losses, we fused TG8 and 422E cells in the presence of Sendai virus. After 2 d of cocultivation at 33° C, the cells were plated in HAT medium at the same temperature. After 3 d, they were shifted to 39° C and kept one more day in HAT medium. After a further 24 h, they were labelled for 2 h with ^3H -uridine, and the 28S rRNA produced was analysed on polyacrylamide gels. This killed most of the mouse cells, which could not grow in HAT medium, while *ts422E* and hybrid cells were unaffected. In this population, production of 28S rRNA can be ascribed chiefly to surviving mouse cells, or mouse-*ts422E* hybrids. Accordingly, the results showed that total production of 28S rRNA was very low and, most important, 16% of this RNA was of hamster origin. In cells treated similarly in the absence of Sendai virus, hamster RNA constituted less than 4% of the total 28S rRNA. Thus, the defect in ribosome production of *ts422E* can be relieved before extensive chromosomal rearrangements in the hybrid cell, as it can be demonstrated as early as 5 d after hybridisation, corresponding to probably less than three generation times.

These findings also prove that the *ts* mutation leading to defective processing of *ts422E* rRNA does not reside in the rRNA itself, for if it did, this RNA would not be susceptible to proper processing even in hybrid cells. It is also interesting that, with respect to the protein(s) involved in rRNA processing, there can be an exchange of products between cells of different species, as mouse gene-product(s) seem able to function in the processing of hamster rRNA.

It has been reported that in mouse-hamster hybrids, when the chromosomes of one species constituted most of the chromosomes of the hybrid, a disproportionately higher percentage of rRNA of that species was present⁸. We do not find this relationship in all our hybrids (Table 2), and at least two (T2 and C16) produce approximately equal amounts of mouse and hamster 28S rRNA. It does not seem likely that this discrepancy is due to different methods of labelling the cells (2-3 h compared with steady state label in ref. 8)

TABLE 2 Chromosomal constitution and production of hamster and mouse rRNA of *ts422E* × mouse cell hybrids

Hybrids	Modal chromosome No.			Total hamster*	% Hamster chromosomes	% Hamster 28S rRNA
	Telocentric	Bi-armed	Total mouse*			
C7	38	56	22	72	76	71
C12	70	58	53	75	58	85
T2	82	48	68	62	47	57
T10	54	29	45	38	46	19
C1	63	33	53	43	45	65
C16	70	58	55	35	39	49

Production of hamster 28S rRNA was calculated as described in Fig. 1 and Table 1.

* Estimated by assuming that the number of hamster telocentrics is proportional to the number of bi-armed chromosomes.

for labelling our hybrid cells for 2 or 24 h did not substantially change the proportion of mouse and hamster 28S rRNA. This discrepancy is more likely to reside in the nature of the hybrid lines examined: we examined ours soon after formation, but Eliceiri⁸ examined hybrids which had undergone extensive propagation and recloning¹². If the production of predominantly one species of 28S rRNA conferred even a slight growth advantage to these cells, this would lead to the selection of stable hybrid lines containing predominantly rRNA of one of the two species.

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Changes in a surface-labelled galactoprotein and in glycolipid concentrations in cells transformed by a temperature-sensitive polyoma virus mutant

In hamster cells transformed by a temperature-sensitive mutant of polyoma virus we have found that the external label in a galactoprotein disappears and the cellular concentration of lactosylceramide increases when the cells are grown at the permissive temperature. These changes can be reversed at the non-permissive temperature.

Three kinds of major membrane changes involving glycoproteins and glycolipids are associated with malignant transformation. They are (1) enhanced agglutinability of cells by some sugar-binding proteins (lectins)¹⁻³; (2) blocked synthesis of gangliosides⁴⁻¹⁰, neutral glycolipids¹¹⁻¹³, fucolipids^{14,15} and some glycoproteins¹⁶ with occasional accumulation of precursor glycans^{4,6,10}; and, (3) enhanced synthesis of sialofucoglycopeptide^{17,18}. Recently a method for surface-labelling cells using galactose oxidase followed by treatment with tritiated sodium borohydride has been used to distinguish between surface galactoproteins of normal and transformed cells¹⁹. A galactoprotein which was labelled in normal hamster NIL cells was not labelled in polyoma-transformed NIL cells, and the introduction of label into this surface moiety seemed to be a correlative of growth control.

The enhanced agglutinability of transformed cells¹⁻³ has been linked directly to the control of cellular multiplication

in two types of transformed cells harbouring thermosensitive mutations. In one case, what is believed to be a mutation in a cellular gene results in the restoration of regulated growth and decreased agglutinability at the restrictive temperature, while the cells seem to be transformed at the permissive temperature^{20,21}. The same phenomenon is observed in cells transformed by thermosensitive polyoma virus mutant ts3^{22,23}.

We have initiated an investigation of the biochemical basis for the thermosensitive changes in the surface membrane of polyoma ts3-transformed BHK cells. Since topoinhibition of DNA synthesis is also rendered thermosensitive in these same cells²², we speculate that the temperature-dependent surface alterations that we have observed may be implicated in the control of DNA synthesis.

BHK cells transformed with temperature-sensitive polyoma virus (BHKpyts3 Cl 7C), those transformed with wild type polyoma virus (BHKpywt Cl 4), and their progenitor cells (BHK Cl 13) were obtained from Dr W. Eckhart at the Salk Institute. Each cell line was grown in Dulbecco's

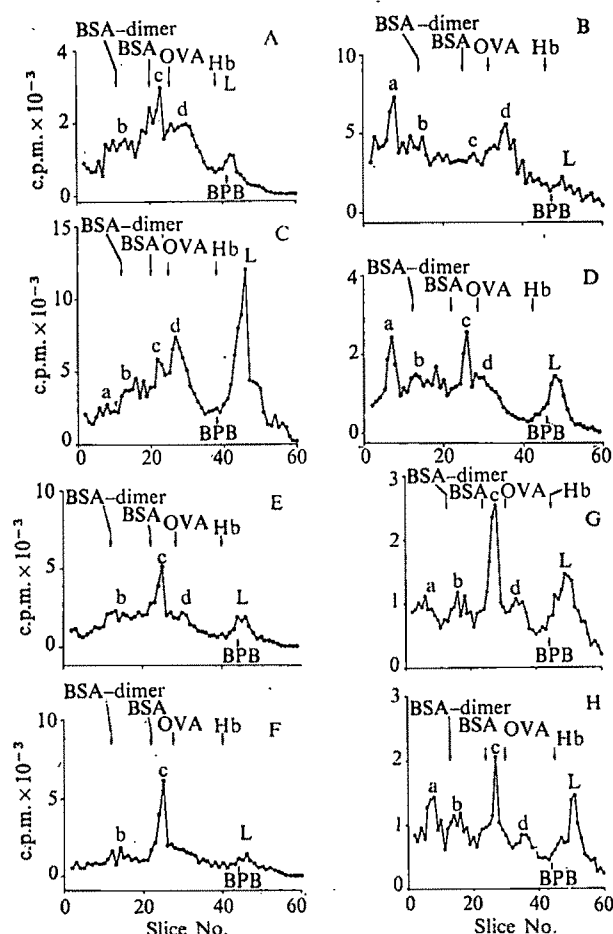


Fig. 1 Profile of external galactosyl label in baby hamster kidney fibroblasts (BHK C13) on acrylamide gel electrophoresis in sodium dodecyl sulphate. Cells were treated on the plates with 10 Worthington units of galactose oxidase for 1 h at room temperature, taken off with EDTA, and labelled as described previously¹⁹. Graphs show subconfluent non-transformed BHK at 32°C (A) and growing at 39°C (C); confluent non-transformed BHK at 32°C (B) and at 39°C (D); wild-type-polyoma-transformed BHK at confluency at 32°C (E) and at 39°C (F) ts3-transformed BHK at confluency at 32°C (G) and at 39°C (H) Acrylamide concentration was 5%; slicing, counting and preparation of internal ¹⁴C-labelled standard proteins were as previously described¹⁹. BSA, bovine serum albumin; OVA, ovalbumin; Hb, haemoglobin; BPB, bromo-phenol blue. Note that galactoprotein a is only pronounced in confluent, non-transformed cells and in BHKpyts3 at non-permissive temperature. Peaks c and d are non-specifically labelled by sodium borotritiate alone. L = lipid label.

TABLE 1 Dependence of galactoprotein a label on cell density in normal BHK cells, cells transformed with wild-type polyoma virus (BHKpywt) and with *ts3*

Cells	Temperature (°C)	Presence or absence of galactoprotein a label		
		Sparse*	Subconfluent†	Confluent‡
BHK normal	32	—	—	++
	39	—	+	++
BHKpywt	32	n.d.	—	—
	39	n.d.	—	—
BHKpyts	32	n.d.	—	—
	39	n.d.	+	++

The apparent molecular weight of galactoprotein a was approximately 200,000. —, Absent; +, obviously present; ++, present in high quantities; n.d., not determined.

* Cells growing separately without touching.

† Cells touching but not saturated.

‡ Cells at saturation density.

modified minimum essential medium containing 10% foetal calf serum at the permissive (32° C) and non-permissive (39° C) temperatures for the *ts3* gene function.

Surface labelling was achieved on cells growing on plastic dishes by the method described previously¹⁹. Glycolipids were extracted with chloroform-methanol and isolated by acetylation: the glycolipids were separated on thin-layer chromatography into fractions lactosylceramide, trihexosylceramide and haematoside. Each fraction was extracted from silica gel with chloroform-methanol-water (1:1:0.1) and the extracts were methanolysed, and the sugars liberated were determined as trimethylsilyl derivatives with myo-inositol as an internal standard by gas-liquid chromatography.

As Table 1 and Fig. 1 show, in cells grown to their maximum saturation density by changing the medium frequently, a surface galactoprotein of BHK with a molecular weight of 200,000 (galactoprotein a) was not labelled in BHKpywt either at 32° C or at 39° C, nor was it labelled in BHKpyts3 at 32° C. However, galactoprotein a of BHKpyts3 was labelled when the cells were grown at 39° C; where they acquired normal morphological appearance, decreased agglutinability with lectins and increased topoinhibition of DNA synthesis.

In actively growing, sparse or subconfluent BHK cells, galactoprotein a was not labelled, whereas it was labelled when growth was inhibited at the saturation density.

As Table 2 shows, in BHKpywt the chemical concentration of lactosylceramide was obviously higher than that of normal BHK cells, and the concentration of trihexosylceramide was considerably reduced. The concentration of lactosylceramide was also sharply increased in BHKpyts3 at 32° C but not at 39° C, whereas the concentration of haematoside was depressed. It is noteworthy, however, that the concentration of trihexosylceramide was quite low at both temperatures in BHKpyts3.

Recently, Hammarström and Bjursell²⁴ observed that isotope incorporation from ¹⁴C-palmitate into trihexosylceramide depends on cell population density and decreased

considerably when the cells were transformed with wild type polyoma virus, in agreement with our previous findings¹⁰. Also consistent with their results, the decreased synthesis of trihexosylceramide in BHKpyts3 at the permissive temperature was not restored at the non-permissive temperature, indicating that decreased synthesis of trihexosylceramide is not essential for expression of certain aspects of the transformed phenotype. Similarly, the growth behaviour in agar and the serum requirement of BHKpyts3 are not known to be temperature-sensitive²², although topoinhibition of DNA synthesis and agglutinability by lectins are temperature-sensitive^{22,23}. Thus, although trihexosylceramide reduction accompanies transformation by polyoma virus, it is not under the control of the *ts3* gene function and therefore does not seem to be implicated in the regulation of cell growth.

A considerable increase of lactosylceramide in some clonal isolates of BHKpywt had also been observed previously⁴. Our results suggest that increased lactosylceramide could be essential for expression of crucial aspects of the transformed phenotype, including the regulation of growth.

The inability to label galactoprotein a in the transformed state shows a good parallel with the appearance of lectin agglutinability, as demonstrated reversibly in BHKpyts3 at permissive and non-permissive temperatures²³. The labelling of the galactoprotein in non-transformed cells at confluency is consistent with an increased reaction of galactosyl residues with *Ricinus communis* protein as observed by Nicolson²⁵. Our surface labelling procedure did not label lactosylceramide of either transformed or non-transformed BHK cells, in agreement with the finding that the lactosyl moiety may not protrude towards the outside of the cells or that the length of the lactosyl moiety is too short to be externally labelled due to steric hindrance.

Perfect reversibility in the labelling of galactoprotein a and the lactosylceramide and haematoside concentrations in BHKpyts3 between permissive and non-permissive temperatures suggest that these membrane parameters are related

TABLE 2 Glycolipid concentration of BHK cells, those transformed with wild-type and with *ts3*

	Temperature (°C)	Glycolipid Concentration $\mu\text{mol per 100 mg dry weight}^*$		
		Ceramide† trihexoside	Ceramide† dihexoside	Haematoside§
BHK normal	32°	0.070	0.218	0.46
	39°	0.075	0.161	0.93
BHKpywt	32°	0.008	0.371	0.77
	39°	0.015	0.329	0.75
BHKpyts	32°	0.040	0.725	0.12
	39°	0.015	0.120	1.21

Concentrations were determined by gas-liquid chromatography.

* Dry weight of the cell residue.

† $\alpha\text{Gal} \rightarrow \beta\text{Gal} \rightarrow \text{Glc} \rightarrow \text{ceramide}$.

‡ $\beta\text{Gal} \rightarrow \text{Glc} \rightarrow \text{ceramide}$.

§ $\text{Sialyl} \rightarrow 2 \rightarrow 3\beta\text{Gal} \rightarrow \text{Glc} \rightarrow \text{ceramide}$.

to growth control in BHK cells. It is possible that deletion of galactoprotein a initiates uncontrolled DNA synthesis, as the loss of this protein has been observed after trypsin treatment of BHK and NIL cells (our unpublished observation), and since trypsin is known to induce the initiation of S-phase, and mitosis²⁶.

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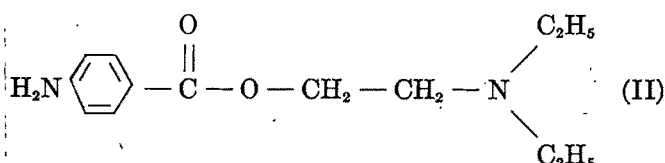
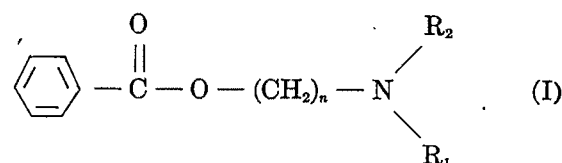
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Enhancement of radiation lethality of *E. coli* B/r by procaine hydrochloride

MANY carbonyl compounds by virtue of the electron affinitive property¹ of their C=O groups are good radiosensitisers in bacterial as well as mammalian cells²⁻⁴. The electronic charge distribution at the carbonyl bond of some local anaesthetics belonging to the class of esters represented by the general formula (I) given below is also known to play an important

role in their anaesthetic action⁵⁻⁸. If these esters are found to be radiosensitisers they may prove very valuable in radiation therapy. Here we present data on one such compound, procaine hydrochloride (II) which is commonly used as a local anaesthetic and has been found to enhance radiation lethality of *Escherichia coli* B/r under anoxia.



E. coli B/r cells grown to stationary phase in nutrient broth (Difco) were suspended in 0.1 M phosphate buffer (pH 7) at a concentration of approximately 2×10^8 cells ml⁻¹. The cell suspension was irradiated in a ⁶⁰Co γ-ray source at a dose rate of 8 krad min⁻¹ and the radiation dose was measured by ferrous sulphate dosimetry. The cell suspension was deoxygenated by bubbling oxygen-free nitrogen before and during irradiation. Sodium nitrate (Analar) and commercially available medical grade nitrous oxide were used as electron scavengers. Procaine hydrochloride (USP) obtained from Hoechst Pharmaceuticals (India) was used without further purification. Toxicity of procaine hydrochloride towards bacteria was determined up to a contact time of 100 min in buffer and only non-toxic concentrations were used in the present studies. Irradiated samples were diluted in sterile phosphate buffer, inoculated into Petri plates and overlaid with nutrient agar (Difco) previously melted and held at 48° C. A minimum of 500 colonies of bacteria were counted at the end of 18 h incubation at 37° C. All the survival curves represent data from experiments repeated at least three times with replicate plates at all dilutions. From the exponential part of the survival curves plotted on a semi log scale, *D*₁ (dose for 1% survival) values were obtained and the dose modification factor (DMF) was calculated as the ratio of *D*₁ in the presence of procaine HCl and that of the control cells.

Procaine HCl at a concentration of 250 μM enhanced the lethality of *E. coli* B/r cells on irradiation under anoxia (DMF = 0.54). It can be seen from Table 1 that a further increase in procaine concentration did not show proportionate decrease in DMF. Whereas procaine HCl was effective also when cells were irradiated under anoxia in nutrient broth, there was no effect if cells treated with the chemical were thoroughly washed before irradiation. Procaine HCl irradiated in aqueous solution under anoxia did not show any toxic effects on unirradiated bacteria indicating that the enhancement of the radiation lethality was not due to some stable toxic products of the anaesthetic. On the other hand, when irradiated cells were immediately mixed (within 1 min of irradiation) with unirradiated procaine HCl there was a significant enhancement in the lethality (Table 1) implicating the inhibition of post-irradiation repair. This would also rule out the involvement of any short-lived transients of the chemical in this process. The fact that the effect of procaine HCl was observed even when irradiation was carried out in the presence of broth or in the presence of an efficient electron scavenger, NaNO₂, at equimolar concentrations (Table 1) further supports this view.

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TABLE 1 Modification of radiation response of *E. coli* B/r by procaine hydrochloride

Medium	Gas	Procaine HCl mM	Other treatment	D ₁ (krad)	DMF
Buffer	N ₂	—	—	74.0 ± 1.4	—
	Air	—	—	24.2 ± 1.1	0.33
	N ₂ O	—	—	68.0	0.92
	N ₂	—	NaNO ₂ (2 mM)	70.0	0.94
	N ₂	0.25	—	40.0 ± 0.6	0.54
	N ₂	0.50	—	40.0 ± 0.6	0.54
	N ₂	2.00	—	37.5 ± 1.2	0.51
	N ₂	10.00	—	36.8 ± 1.6	0.50
	N ₂	25.00	—	30.9 ± 1.0	0.42
	N ₂	25.00	Chemical washed off before irradiation	74.0	1.00
	N ₂	25.00	Chemical washed off after irradiation	31.2 ± 1.3	0.42
	N ₂	25.00	Irradiated cells mixed with unirradiated procaine HCl	38.5 ± 1.5	0.52
	N ₂	2.00	NaNO ₂ (2 mM)	38.0	0.51
	Air	25.00	—	26.0 ± 2.0	0.35
	N ₂ O	25.00	—	66.5 ± 1.5	0.90
Nutrient broth	N ₂	—	—	87.0 ± 7.0	—
	Air	—	—	28.0 ± 2.0	0.32
	N ₂	25.00	—	43.5 ± 1.5	0.50
	Air	25.00	—	28.0 ± 2.0	1.00

Whereas the actual mechanism still remains uncertain, procaine HCl was not found to react with cysteine (our unpublished results) as studied by Ellman's reaction modified by Butterworth *et al.*⁷ This could explain the result that cells treated with procaine HCl but irradiated after being thoroughly washed were not affected. The local anaesthetics and procaine HCl in particular, are known to interact with cellular membranes⁸ leading to various changes in membrane characteristics^{9,10}. Since membrane is believed to play an important role in the modification of radiation response of cells^{11,12} it is likely that the enhancement of radiation lethality by procaine HCl may be somehow related to its interaction with the bacterial membrane. The enhancement of damage by N₂O (ref. 13) (Table 1) could also be attributed to its anaesthetic action although alternative mechanisms¹⁴ have been suggested. Shortlived transients do not seem to play any significant role and so the observation that N₂O₂, which is also an electron scavenger, considerably reduced the effect of procaine HCl (DMF = 0.90) indicates a competition between the two anaesthetics for sites to interact with the cellular membrane, rather than with the radiolytic transients of water.

The ineffectiveness of procaine HCl under oxic conditions of irradiation still needs a satisfactory explanation. The following facts, however, must be kept in mind while suggesting any mechanism for this effect: (i) free radical intermediates may not be playing any role in enhancement of damage by procaine HCl; (ii) oxygen itself enhances radiation effects implicating the cellular membrane¹⁵; (iii) the radiation damage caused in presence of oxygen is generally non-repairable¹⁶.

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Demonstration in mouse of X-ray induced deletions for a known enzyme structural locus

THE known lethal alleles at the albino locus of mice are radiation-induced and cause prenatal or early neonatal death. Four alleles expressed in the homozygous newborn as absence of pigment and early neonatal death (c^{1408} , c^{112K} , c^{65K} , and c^{2H}) were found to cause hypoglycaemia and deficiency of glucose-6-phosphatase and other enzymes¹⁻³. Two additional radiation-induced alleles (c^{6H} and c^{25H}) act as early embryonic lethals. Complementation tests between these lethal-albino alleles suggested that all six mutations could be deficiencies for genetic material other than just the albino locus⁴. Since X rays are known to produce small deletions^{4,5}, all six of these mutations have been tested with various closely linked genes using the standard deletion mapping procedure. Unfortunately, the two most closely linked genes to c , $taupe$ (tp) and shaker-1 ($sh-1$) (order with centi-Morgans (cM) distance, $tp - 2 - c - 4 - sh-1$), seemed not to have been deleted in any of the six lethal-albino mutations^{2,6}. Recently,

TABLE 1 MOD-2 phenotypes of individuals produced by crossing c^{ch}/c^* to SM/J mice.

Genotype of original c^{ch}/c^* individual	^a Untyped F ₁ offspring		^b Typed F ₁ offspring			
	MOD-2AB	MOD-2A	$c^{ch}/+$ MOD-2AB	MOD-2A	$c^*/+$ MOD-2AB	MOD-2A
$c^{14COS} Mod-2^?$ $c^{ch} Mod-2^b$	11	0	2	0	2	0
$c^{6H} Mod-2^?$ $c^{ch} Mod-2^b$	4	5	3	0	0	5
$c^{25H} Mod-2^?$ $c^{ch} Mod-2^b$	3	3	5	0	0	5

the gene mitochondrial malic enzyme (*Mod-2*) that codes for electrophoretic variants of mitochondrial malic enzyme (MOD-2: EC 1.1.1.40, L-malate: NADP oxidoreductase, decarboxylating) was shown to reside in chromosome 7 one cM from *c* between *c* and *sh-1* (ref. 7 and E. M. E. and D. L. Coleman, in preparation). We present evidence here showing that two of these mutations (c^{6H} and c^{25H}), but not the c^{14COS} mutation, are deletions that extend into the *Mod-2* locus.

Stocks of each of the lethal-albino alleles are maintained by brother-sister mating of heterozygous individuals containing the chinchilla (c^{ch}) allele at the *c* locus and one of the lethal-albino alleles (c^*). At the time of weaning, the phenotype of homozygous chinchilla (c^{ch}/c^{ch}) is darker than that of heterozygotes for chinchilla and albino (c^{ch}/c^*). In the crosses reported here, the normal *c* allele (+) was obtained from randomly-bred Swiss-Webster mice. The *Mod-2*^a allele was obtained from the SM/J strain maintained at the Jackson Laboratory. The lethal-albino stocks and the Swiss-Webster stock carried only the *Mod-2*^b allele.

Individual hearts were homogenised in one to two volumes of distilled water and then Triton X-100 was added to give approximately a 1% final concentration. The supernatant was prepared by centrifuging the homogenate at 20,000 *g* for 15 min and applied directly to the gel wells. Electrophoresis was performed in a Buchler Vertical Starch Gel Apparatus, cathode to anode, at 150 V for 18 to 20 h. The bridge buffer consisted of 0.233 M Tris base, 0.086 M citric acid (pH 7.0). The gel consisted of 12% Electrostar in a gel buffer of 0.0043 M Tris base, 0.0016 M citric acid (pH 7.0). The gel was sliced and stained according to the method of Shows *et al.*⁸

Individual c^{ch}/c^* mice (MOD-2B),¹ where c^* represents either the c^{6H} , c^{25H} , or c^{14COS} allele, were crossed to SM/J

mice (MOD-2A) and the *Mod-2* phenotype of individual offspring was determined (Table 1, Column a). In the cross involving c^{14COS} only one type of offspring was obtained, namely that with the heterozygous (*Mod-2*^a/*Mod-2*^b) phenotype, MOD-2AB. But in the crosses involving either c^{6H} or c^{25H} , segregation of two types of offspring occurred: those with the heterozygous (*Mod-2*^a/*Mod-2*^b) phenotype, MOD-2AB, and those that displayed the MOD-2A phenotype characteristic of the SM/J strain (Table 1). This raised the suspicion that perhaps individuals carrying one of the two lethal-albino alleles, c^{6H} or c^{25H} , might lack the *Mod-2* locus in *cis* position with c^* accounting for the lack of expression of the *Mod-2b* allele. This hypothesis was put to test by progeny testing these F₁ individuals derived from crosses of c^{ch}/c^* (MOD-2B) by $+/+$ (SM/J, MOD-2A). Crosses of these to a Swiss Webster albino stock (*c/c*), permitted the identification of the particular allele, c^{ch} or c^* , at the albino locus. Only those F₁s which carried c^{6H} or c^{25H} lacked the *Mod-2b* allele whereas this was present in all those F₁s carrying the c^{ch} -allele (Table 1, Column b and Fig. 1).

We believe this is the first demonstration in mammals of X-ray induced deletions for an identified enzyme structural locus. The c^{6H} and c^{25H} deletions extend in length at least 1 cM, since *Mod-2* is involved. Our data are also consistent with the genetic complementation map constructed for the six lethal-albino mutations where c^{6H} and c^{25H} complement with c^{14COS} but not with each other⁹. The data suggest that mitochondrial malic enzyme may be essential in embryonic development as the two lethal albino alleles in which its structural locus is deleted are early embryonic lethals.

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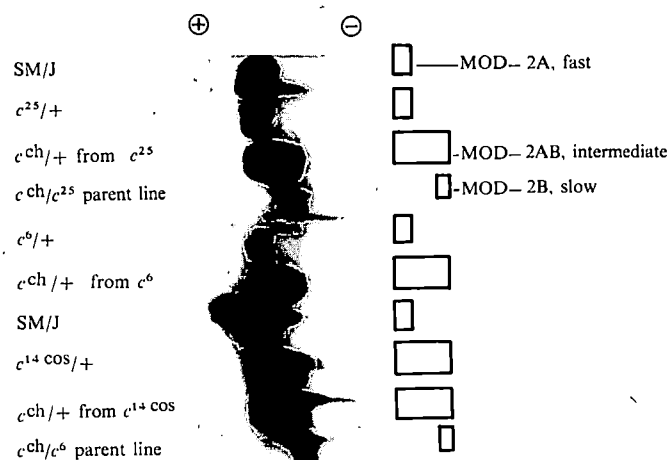


FIG. 1 The MOD-2 phenotypes of SM/J, lethal-albino stocks, and lethal-albino X SM/J F₁ mice whose genotypes were determined by a cross to Swiss-Webster mice. Heart extracts were run on starch gel electrophoresis.

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Gene dosage in a deletion for a nuclear-coded, mitochondrial enzyme

It is a general principle of mammalian biochemical genetics that enzymatic activity is proportional to gene dosage; that is, co-dominance of the biochemical phenotype is usual in heterozygotes. A well studied exception is the lethal albino locus of mice. The radiation-induced lethal albino alleles cause prenatal or early neonatal death. The mutations are unusual in their lack of gene dosage effect. Four alleles (c^{1400} , c^{112K} , c^{65K} , c^{3H}) that are expressed in newborns as absence of pigment, hypoglycaemia, and neonatal death are associated with glucose 6-phosphatase deficiency^{1,2}. Heterozygotes distinguishable by pigment dilution have the same glucose 6-phosphatase levels as normal homozygotes in a number of situations, including enzyme induction with corticoids, glucagon, dibutyryl cyclic AMP, by producing alloxan diabetes, and during normal post-natal development^{3,4}. Four other enzymes (tyrosine aminotransferase, serine dehydratase, bilirubin glucuronyl transferase, and spectrophotometrically-determined cytochrome P-450) that are deficient in lethal albino homozygotes are present in normal levels in heterozygotes (refs 3-5 and M. M. Thaler, R. P. E., and S. Gluecksohn-Waelsch, in preparation). There is evidence that mutation of something other than structural genes for the enzymes is involved. Residual amounts of glucose 6-phosphatase and tyrosine aminotransferase are present in lethal albino homozygotes. Complementation of these lethal albino alleles with two additional lethal albino alleles, c^{6H} and c^{25H} obtained from Harwell, resulted in normal enzyme levels^{4,5}. Also, abnormalities of endoplasmic reticulum of lethal albino hepatocytes are strikingly evident on electron microscopy⁶. Enzymes deficient in homozygotes are either cyclic AMP-induced or microsomal. A mutation in the gene for a membrane structural protein or in the gene for a component of the cyclic AMP induction system has been suggested as an underlying defect which might account for the deficiency of a number of enzymes and the failure to demonstrate heterozygote dosage effect, although biochemical and immunological techniques have not yet detected any abnormality (R. P. E., P. Siekevitz, K. Jacobs, and S. Gluecksohn-Waelsch, in preparation).

It has been demonstrated that in two lethal albino alleles (c^{6H} and c^{25H}) which cause early embryonic death the deletion extends to the adjacent structural locus for mitochondrial malic enzyme (*Mod-2* EC 1.1.1.40, L-malate NADP oxidoreductase, decarboxylating)⁷. We compared malic enzyme activity in hearts and kidneys of lethal albino heterozygotes, which possess one copy of the structural gene, to their normal littermates, which possess two copies. A 2:1 ratio of enzyme activity is expected if dosage holds true. Typed offspring of the c^{6H}/c^{6H} or $c^{6H}/c^{25H} \times SM/J (+/+)$ had been tested by the cross to Swiss Webster (c/c)⁷. These

typed animals were either hemizygous (*Mod-2a*) or heterozygous (*Mod-2ab*) at the structural locus for mitochondrial malic enzyme.

Adult mice were killed by cervical dislocation. All operations were carried out at 0° C. 73% of malic enzyme in mouse hearts is mitochondrial⁸. Homogenates of individual hearts were prepared by homogenising minced tissue in 0.5 ml of distilled water. Triton was added to the homogenate to make a 1% solution, which was incubated for 15 min and then centrifuged at 700g for 30 min. The supernatant was collected for enzyme assay and protein quantification.

Kidney mitochondria were purified by homogenising the kidneys from individual animals in three volumes of 0.25 M sucrose in 0.01 M, pH 8 glycylglycine. The homogenate was centrifuged at 700g for 10 min. The supernatant was collected and spun at 8,700g for 15 min. The resulting pellet was resuspended in 1.0 ml of sucrose-glycylglycine and Triton was added to make a 1% solution. After incubating for 15 min, the preparation was centrifuged at 39,000g for 20 min and the supernatant saved for assay and protein quantification.

Assay for malic enzyme activity was carried out with a spectrophotometer measuring change in optical density at 340 nm at 37° C. The total volume was 0.2 ml and final concentrations of the reagents were: glycylglycine, 0.2 M; MnCl₂, 0.79 mM; NADP, 2 mM; and, L-malic acid, 16.5 mM. Malic acid was added to start the reaction after the other reagents and an appropriate dilution of enzyme preparation had equilibrated at 37° C for 5 min. Protein concentrations of the enzyme preparations were determined by Lowry's method which was not affected by the low concentrations of Triton X-100 present.

As seen in Table 1, about half as much malic enzyme was found in hearts from the hemizygous mice as was found in normal, heterozygous mice. The difference is highly significant. In fact, there is a better fit to the 2:1 ratio than is expected for material where only 73% of the enzyme is mitochondrial. Given the standard errors, the activity ratio is not greatly different than the expected 63.5:100. A significant difference was however not found with the purified kidney mitochondria although the trend is in the expected direction. The difference is not due to a different specific activity of the MOD-2A enzyme (the fast form solely present in the hemizygous mice) as *Mod-2a* homozygotes (SM/J strain) showed 35.0 ± 4.4 nmol per min per mg protein in hearts and 54.6 ± 5.6 nmol per min per mg protein in purified kidney mitochondria.

We conclude that the principle of gene dosage for enzymatic activity in heterozygotes also applies for a deletion in the structural gene of mitochondrial malic enzyme. This is in contrast to the failure to observe dosage effects in lethal albino heterozygotes with glucose 6-phosphatase and a number of other enzymes that are deficient in lethal albino homozygotes. It seems likely that these exceptions to gene dosage are related to a defect in a structural component, for example, microsomal membrane protein or in regulation rather than mutation of structural genes. Thus, gene dosage in mammals cannot always be explained by normal levels of enzymatic protein of which half is nonfunctional (but which might often be detected as CRM as has been found with human gal-1-P uridyl transferase deficiency⁹).

It is interesting that gene dosage is present for a nuclear coded mitochondrial enzyme. In yeast and *Neurospora*, blockage of mitochondrial transcription and translation stimulates synthesis of nuclear coded mitochondrial enzymes, suggesting that mitochondria make a repressor(s) regulating nuclear gene expression¹⁰. The finding that the deletion of one nuclear gene for a mitochondrial enzyme leads to a halving of the enzyme's activity would suggest that activator controls, if present in mammals, are not locus specific. The detection of human, nuclear controlled, mitochondrial en-

TABLE 1 Malic enzyme activity in mice with a deletion for mitochondrial structural gene (*Mod-2*) and normals

	nmol/min/mg protein	
Tissue genotype:	$c^6/+$ or $c^{25H}/+$	$c^{6H}/+$
heart homogenate	(6) $18.0 \pm 1.5^*$ $t = 4.58, P < 0.01$	(5) 39.4 ± 3.9
kidney mitochondria	(6) 46.6 ± 4.3 $t = 1.97, P = 0.1-0.05$	(5) 59.8 ± 5.1

* (No. of animals) mean \pm s. e.

zymes in putative mouse mitochondria in man-mouse somatic cell hybrids¹¹ might also suggest that such controls are not species specific. Mammalian nuclear-mitochondrial interactions may involve different control mechanisms.

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Note added in proof: E. M. Eicher and D. L. Coleman (personal communication) find dosage for *MOD-2* in the trisomic situation using the flected translocation.

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Amelioration of Marek's disease and absence of vaccine protection in immunologically deficient chickens

MAREK's disease (MD) is a lymphoproliferative disease of chickens which is generally considered to be neoplastic¹. It is probably the most commonly occurring neoplasm of any animal and until recently it caused extensive economic loss, estimated to be in excess of 150 million dollars annually, to the poultry industry². Several viral vaccines which will effectively prevent the disease have been developed³ and the most extensively used is a herpesvirus isolated from turkeys (HVT)⁴, which will prevent the disease in laboratory and field trials^{5,6}.

The characteristics of the protection are unusual. On vaccination, chickens develop persistent viraemia with HVT.

Also, vaccination with this virus does not prevent infection of the host with virulent MD virus (MDV), replication and persistence of the virulent virus in the host, and shedding of the virulent virus from the host^{6,7,8} albeit at a slightly lower level. Thus, the protection cannot be mediated entirely by immune mechanisms directed against virus replication and shedding. A variety of mechanisms have been proposed⁹⁻¹².

Cells constituting MD lymphomas could have originated from bursa, thymus or bone marrow¹. From antigenic marker studies, they are apparently predominantly thymus-derived lymphocytes^{12,13}. Also, neo-natal bursectomy does not interfere with MD induction¹⁴, and thymectomy decreased the proportion of genetically susceptible birds that died of MD or had MD lymphomas¹. The thymus cells of the tumour may have two roles: as a source of proliferative target cells or as a mediator of cellular immune response against the target cells. We show here that cyclophosphamide (CP) treatment significantly reduces the incidence of MD. Also, immunologically deficient chickens bursectomised with high doses of CP were not protected against MD by vaccination with HVT.

Chickens, highly susceptible to MD, were line 7 in experiment 1 or a cross between line 15 males and line 7 females in experiment 2 (ref. 15). The parent flocks are known to have antibody to MDV that is transmitted passively to the progeny. Progeny hatched and reared in isolation, however, are free of MDV and do not acquire antibody to MDV. All chicks were reared in modified Horsfall Bauer isolators. For virus isolation, approximately 2×10^7 spleen cells were inoculated on confluent DEF cultures prepared and maintained as described¹⁶. Plaques were counted 14 d after inoculation. Antibody to MDV was assayed by the agar gel precipitation test with antigen prepared in duck embryo fibroblasts¹⁷ or by the indirect fluorescent antibody test¹⁸.

To test for the ability of the chickens to produce antibody to sheep erythrocytes (SE), we inoculated groups of chickens at 4, 6 or 7, and 9 weeks of age with 1×10^8 to 2×10^8 erythrocytes at each of five sites per chicken. Serum was collected at the end of the experiments (8 weeks for experiment 1 and 11 weeks for experiments 2 and 3), and antibody was assayed by haemagglutination¹⁹. All birds dying during the experiment and all those that survived were examined for MD lesions by gross and histopathological methods²⁰. For chemical bursectomy, chicks were inoculated intramuscularly on the first 4 d of life with either 4 or 5 mg of CP d⁻¹ (Cytosan, Mead Johnson Laboratories, Evansville, Indiana). In previous experiments (H.G.P., unpublished), chicks of line 15 \times 7 inoculated with a total of 16 mg of CP were unable to produce antibody to SE, *Brucella abortus*, and bovine serum albumin, and most of the chicks were agammaglobulinaemic. The HVT strain FC126 in its eleventh duck embryo fibroblast passage⁴ was administered on day 6. The JM strain⁵ and cloned preparation of the GA strain²¹ of MDV, which were highly pathogenic, were used to induce

TABLE 1 Effect of cyclophosphamide on Marek's disease (Experiment 1).

CP	Treatment MDV	No. of Chickens	% Marek's disease response			Virus isolation	Antibody to:	
			Dead*	Gross†	Total‡		MD	SE
16 mg	7 d	8	38	50	100	3/3	0/3	
16 mg	—	7	0	0	14§			0/7
—	7 d	12	67	83	92	1/4	4/4	
—	—	14	0	0	14§		0/5	13/13

CP, Cyclophosphamide administered intramuscularly, 4 mg d⁻¹ for the first 4 d after hatching; MDV, Marek's disease virus, JM strain, inoculated intra-abdominally, 1,100 plaque-forming units (PFU) per chick at 7 d of age; SE, sheep erythrocytes injected intramuscularly (10^8 cells per chick) at 4 and 6 weeks of age and tested at 8 weeks. Antibody to SE was measured by haemagglutination. Antibody to MDV was measured by the indirect fluorescent antibody test.

* % Chickens dying of MD.

† Dead plus survivors that had gross MD lesions at the end of the experiment at 8 weeks of age.

‡ Gross plus survivors that had microscopic MD lesions.

§ Chickens in these groups had minor histological lesions indistinguishable from those caused by MDV.

TABLE 2 Effect of cyclophosphamide on Marek's disease and on protection offered by the herpesvirus of turkeys (Experiment 2).

CP	Treatment		No. of Chickens	Dead*	% Marek's disease response		Total†
	HVT	MDV			Gross‡		
20 mg	6 d	3 weeks	10	50	50		90
20 mg	—	3 weeks	16	31	38		69
20 mg	—	6 d	9	33	67		89
20 mg	—	—	11	0	0		0
—	6 d	3 weeks	10	0	0		0
—	—	3 weeks	12	42	67		92
—	—	6 d	15	67	80		100
—	—	—	14	0	0		0

CP, Cyclophosphamide administered intramuscularly, 5 mg d⁻¹ for the first 4 d after hatching; HVT, the herpesvirus of turkeys inoculated intra-abdominally, 3,700 (PFU) per chick at 6 d of age; MDV, Marek's disease virus, GA strain, inoculated intra-abdominally, 33,000 (PFU) per chick at 6 d of age or 56,000 (PFU) per chick at 3 weeks of age. Experiment ended at 11 weeks of age.

*, †, ‡ See Table 1.

MD by inoculation on the sixth to seventh day of age or to challenge immunity by inoculation on day 21.

Treatment with 16 mg (Table 1) or 20 mg (Tables 2 and 3) of CP significantly reduced mortality ($P \leq 0.01$ when data from both times of exposure are pooled and compared in a one-tailed χ^2 test) and gross lesions ($P \leq 0.01$) of MD even when MD virus was given 3 weeks after CP treatment (Tables 2 and 3). When histological lesions were included, the differences were not significant. The CP-treated chickens tested were infected with MDV as the virus was isolated from most birds tested (Table 3) and most had MD lesions. None of those tested could respond with antibody to either viral antigens or SE. They were therefore deficient in bursa-dependent lymphoid functions.

Experiments 2 and 3 were designed to determine whether CP would affect protection by HVT. In both experiments, vaccinated birds not treated with CP, whose immunity was challenged with the GA (Table 2) or JM (Table 3) strains of MD at 3 weeks of age, were fully protected, those treated with CP had up to 90% MD, depending on the criteria used to measure the response. Thus, these chickens lacked the immunity to MD conferred on normal chickens by HVT. Virus re-isolated from both CP-treated and untreated chickens at the end of the experiment showed that they had become infected with HVT. Although some chickens died, most survived. These surviving chickens were unable to produce antibody to viral antigens. We conclude that they were deficient in their bursa-dependent lymphoid functions.

Among the two control groups not inoculated with MDV (Table 1) and one group inoculated with HVT (Table 3), 1, 2, and 2 birds, respectively, had mild lymphoid infiltrations in the nerves. This type of lesion has been described previously in isolated controls^{7,18,22} and is considered to be unrelated to MDV infection. The presence of viral plaques in cultures from two groups of birds that had not been inoculated with either MDV or HVT (Table 3) cannot be

explained because these groups of birds also lacked antibody to viral antigens.

Treatment with CP eliminated bursa-dependent immune functions and caused a temporary lymphoid depletion of the thymus²³. By 15 d, however, the appearance of the thymus had returned to normal, and at 1 month (the earliest time tested) it was functionally competent. Because surgical bursectomy and irradiation did not affect MD¹⁴ and because bursa-dependent lymphoid cells were only a small part of MD lymphomas^{12,13}, it was unlikely that the effect of CP on the bursa would have been responsible for the observed reduction in MD. Because thymectomy reduced MD lymphomas in susceptible chickens, the effect of CP on the thymus may possibly have accounted for the reduction in MD, particularly because a high dose of CP was used. Prolonged reduction of thymus-dependent function was not observed²³ however. Tests of thymus function during the development of MD in CP-treated chicks would be helpful in determining the role of the thymus. Also CP may possibly have affected bone marrow cells which may be involved in the pathogenesis of MD (ref. 1). A combination of these effects may also have occurred.

Protection against MD by HVT was largely absent in CP-treated chickens even though immunity was challenged when birds were 3 weeks old. The absence of protection was most likely due to the obliteration of bursa-dependent lymphoid cell function by CP although CP may also have had an effect on thymus function. The effect of CP on thymus function was not sufficient to prevent MD completely; some thymus target cell function or cellular immune response to target cells may have persisted. The role of the thymus-dependent immune system in immunity and the effect of CP on it are therefore still undetermined. If the bursa-dependent lymphoid system played a major role in protection induced by HVT vaccine, its effect was probably mediated through antibody to the tumour cell or virus-infected cell rather than through

TABLE 3 Effect of cyclophosphamide on Marek's disease and on protection offered by the herpesvirus of turkeys (Experiment 3).

CP	Treatment		No. of Chickens	Dead*	% Marek's disease response			Virus isolation	Antibody to:	
	HVT	MDV			Gross‡	Total‡			MD	SE
20 mg	6 d	—	11	0	0	20§		5/7	0/7	
20 mg	6 d	3 weeks	11	0	45	55		6/7	0/7	
20 mg	—	3 weeks	6	17	50	83		5/6	0/6	
20 mg	—	—	7	0	0	0		1/7¶	0/7	0/7
—	6 d	3 weeks	10	0	0	0		6/7	7/7	
—	—	3 weeks	14	57	86	93		5/6	6/6	
—	—	—	10	0	0	0		1/7¶	0/10	10/10

CP, Cyclophosphamide administered intramuscularly, 5 mg d⁻¹ for the first 4 d after hatching; HVT, the herpesvirus of turkeys inoculated intra-abdominally, 4,000 PFU per chick at 6 d of age; MDV, Marek's disease virus, JM strain, inoculated intra-abdominally, 690 PFU per chick at 3 weeks of age; SE, sheep erythrocytes injected intramuscularly, 5×10^8 cells per chick at 4 and 6 weeks of age. Antibody to SE was measured by haemagglutination. Antibody to MDV was measured by the agar gel precipitation test. The experiment ended at 11 weeks of age.

*, †, ‡ See Table 1.

§ Both chickens had minor histological lesions indistinguishable from those caused by MDV.

¶ Plaques in one of two replicates possibly due to procedural error.

antibody neutralising the virus. This is because cell-free virus accessible to neutralisation by antibody cannot be demonstrated in blood and internal tissues of MDV-infected chickens. The absence of an effect of vaccine-induced immunity on infection, replication, and shedding of the virulent virus from the host may also have resulted from the highly cell-associated nature of MDV within the body of the chicken. Nevertheless, we conclude that an immunosuppressive drug will eliminate HVT vaccine protection against MD.

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Are non-dividing cells present in ageing cell cultures?

THE idea that an increased number of non-dividing cells accumulate during the growth decline of human embryonic fibroblasts (HEF) cultivated *in vitro* has been put forward¹. This concept of a non-dividing fraction of cells seems to be widely accepted^{2,3}, but no compelling evidence exists so far, to show that non-cycling cells exist in old cultures of human fibroblasts. On the contrary, it seems that most, if not all, the cells can divide up to the end of the lifespan of human

diploid cell lines. What seems to take place, however, is an increased transit time and heterogeneity of cell cycles^{4,5}.

The first experiments that analysed the kinetics of cell division of human fibroblasts at different passage levels^{4,5} showed a decrease in the number of cells which synthesised DNA during a 24 h labelling period and after a continuous labelling between subcultivation and confluency. This seemed to be caused by a prolongation of the generation time due to a delay in the G₁ and G₂ periods of the division cycle. It should be stressed that these experiments referred only to the time between a subcultivation and confluency and did not consider the possibility that cells which did not divide during one passage might do so in the following one. It was subsequently reported⁶ that the percentage of cells capable of division decreased with increasing age in tissue culture. The latter experiments, however, were done by plating 200 cells per 60 mm Petri dish so that each cell remained isolated, so it reveals the number of cells capable of dividing when cloned. This is completely different from how a cell behaves when in the proximity of other cells. It has also been shown⁷ that the percentage of cells capable of entering DNA synthesis after a 30 h labelling period at each passage of human diploid cell lines declines as a function of either time or population doublings. This technique also allows the evaluation of the age of the culture, which is much more precise than referring to the passage number.

These reports⁴⁻⁷ form the basis for the concept that non-cycling cells are present in increased amounts during cell senescence *in vitro*. The cells that did not synthesise DNA were not followed^{4,5,7} in subsequent passages however, and Merz and Ross⁶ analysed cell division in an environment unfavourable to the cell.

When phase II (20th passage) HEF were subcultivated with different split ratios, (cells from one flask were subcultivated by transfer into either another flask (1:1), into two flasks (1:2), or into 4 flasks (1:4)), and were labelled continuously, 21% of the cells synthesised DNA in the first group, 75% in the second, and 97% in the third group (Table 1). This shows that with higher inocula (1:1 and 1:2 splits) not all the cells have time to enter DNA synthesis before growth stops. After a 1:4 split however, almost all cells had time to enter the division cycle before they were affected by the mechanisms responsible for the inhibition of division during cell crowding. Thus, the results of an experiment with only a 1:2 split (which is the way these cells are usually carried) would have suggested that at this passage there are 25% non-dividers.

When HEF very near the end of their lifespan *in vitro* are labelled continuously with tritiated thymidine (³H-TdR) from the time of seeding to the time when further growth ceases, and are analysed by autoradiography, 66% of the nuclei become labelled during that time (Table 2). If identical cultures are subcultivated after that period and are again labelled continuously with ³H-TdR, 7 d after the second subcultivation 86% of the nuclei are labelled and 2 weeks later 92% of the cells are labelled. (The cultures were confluent from 17 d.) Thus only 8% of the cells remained unlabelled after the two last passages of this HEF line. This cannot be due to dilution of the non-dividers by dividing cells, for even if all the cells that had entered DNA synthesis

TABLE 1 Percentage of cells which synthesised DNA between subcultivation and resting phase after different split ratios

Split ratios	Percentage
1:1	21
1:2	75
1:4	97

Cultures were labelled continuously during 4 days with 0.01 μ Ci ml⁻¹ ³H-TdR (specific activity 1.9 Ci mM⁻¹)

TABLE 2 Percentage of cells which synthesised DNA during the 45th and 46th subcultures of an HEF cell line

Days after subcultivation when duplicate cultures were fixed	Cells	
	Between the 45th subculture and confluency (%)	Between the 46th subculture and confluency (%)
11	66	
7		86
11		86
14		92
17		94
24		92
29		92

Cultures were labelled continuously by adding 0.01 $\mu\text{Ci ml}^{-1}$ specific activity 1.9 Ci mM^{-1} every 4d.

had divided, the non-dividing fraction would be diluted to 20%. Also, it was previously shown^{4,5} that in late passages the population does not double because many of the cells that enter DNA synthesis take a long time to reach mitosis, because they are delayed in the G_2 period.

With chick fibroblasts, nearly 100% of the cells divide between each subcultivation and confluency, in human fibroblasts however, a large proportion of the cells do not enter DNA synthesis during one passage, but do so during the following passage. This difference between human and chick cells could be explained on the basis of the increased heterogeneity of the cell cycles in human cells. As a result of this heterogeneity of generation times, when human cells reach saturation density some cells divide one or two times (short cycles) and others do not have time to complete their cycle⁹. In old populations, since this heterogeneity is more pronounced⁴, more cells are unable to complete the cycle before saturation density is reached. In chick fibroblasts, however, although the generation time also seems to be prolonged⁸, it is perhaps uniformly prolonged throughout the whole population, so that at each passage every cell has an equal chance of completing the division cycle before growth stops.

Whatever the reasons, in the systems studied so far, non-cycling cells do not seem to be a feature of cell ageing *in vitro*.

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Androgen receptors in a Syrian hamster ductus deferens tumour cell line

We have established in culture a cloned line of hamster ductus deferens cells that contains a saturable, high affinity testosterone receptor protein. These cells exhibit an increased rate of DNA synthesis in response to testosterone and 5 α -dihydrotestosterone (DHT) treatment, although they probably do not require either androgen for survival. We believe

this is the first description of a cloned cell line that exhibits a growth response to a physiological dose of androgen. The mechanism of action of the steroid may be similar to that in the normal tissue of origin.

A transplantable leiomyosarcoma (Accession No. 14072, Experimental No. LX-7330-MU9-TB-MU1) from Syrian hamster ductus deferens tissue was obtained^{1,2} in March 1971. The original tumour from which the DDT₁ cells were established was induced in male hamsters by long-term administration of diethylstilboestrol (DES) and testosterone³. The tumour was autonomous when received but showed some T-binding activity (J. S. N., unpublished observation). The sarcoma was dispersed by trypsinisation and placed in culture in Ham's F-10 with 10% horse serum, 5% foetal calf

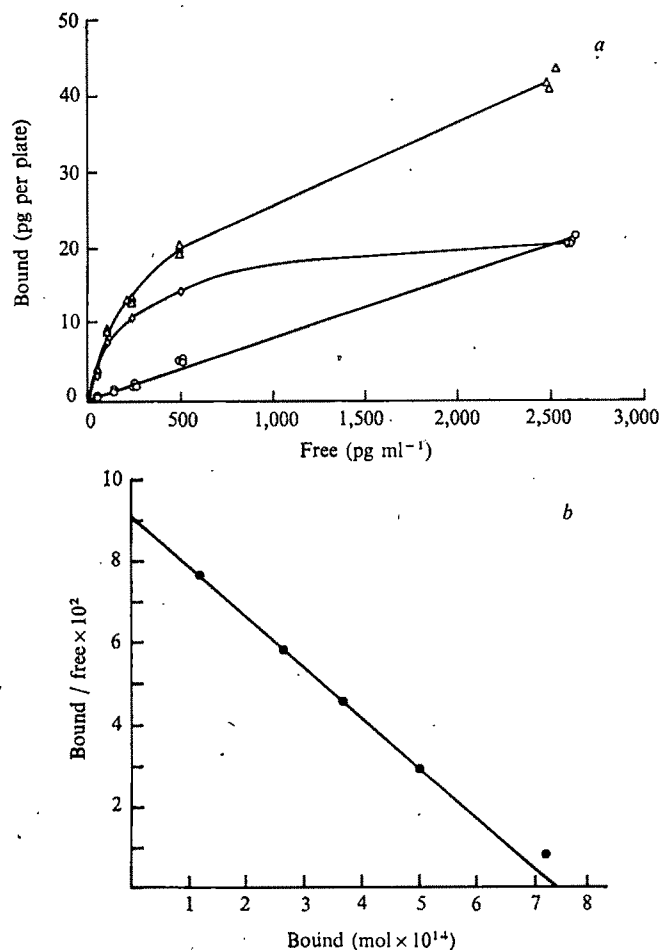
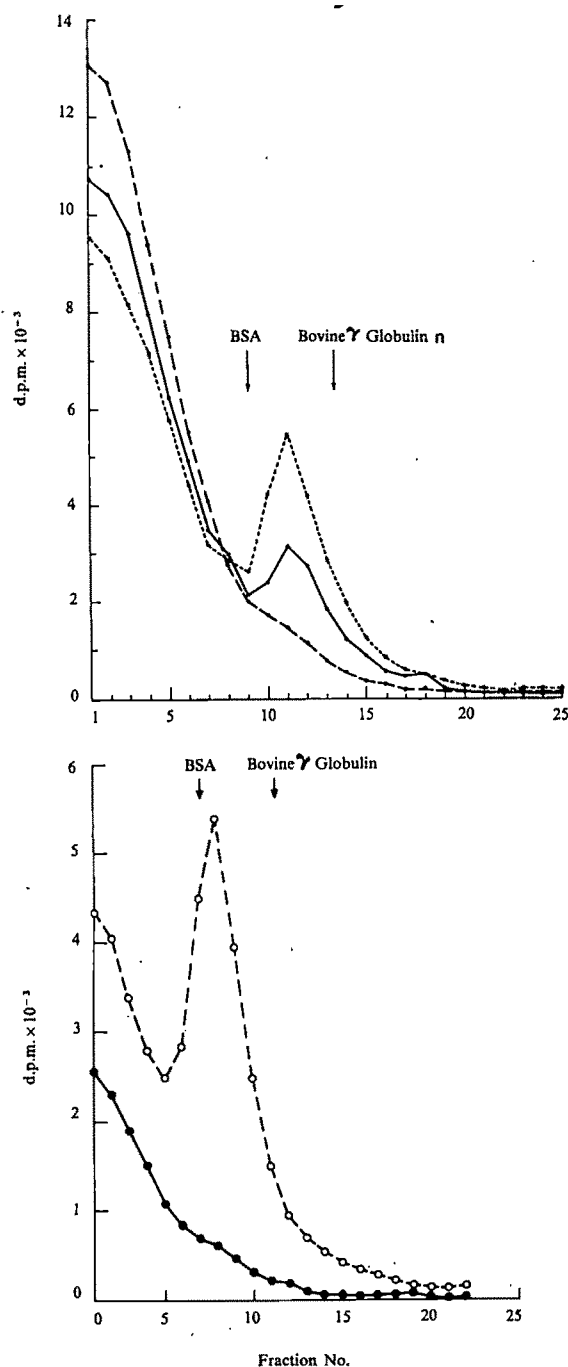


Fig. 1 a, Cells grown in roller culture were collected by shaking, pelleted at 800g, and washed 2 h in minimal essential medium (MEM) buffered with HEPES pH 7.6 at 4° C. Aliquots of cells in 10 ml of MEM were distributed evenly to 25 ml Erlenmeyer flasks and warmed to 37° C. 1, 2, 6, 7 ^3H -testosterone (Specific activity, 91 Ci mmol^{-1} New England Nuclear) dissolved in ethanol-MEM 1:1 was added at varying concentrations either alone or in combination with fifty-fold excess testosterone. At no time did the ethanol exceed 0.1%. Incubation was terminated at 75 min by withdrawing samples and immediately placing them on ice (all further operations are at 4° C unless indicated). All samples were centrifuged at 1,400g and washed twice with Tris saline pH 7.6. Bound ^3H -testosterone was extracted into 3 ml of absolute ethanol (at 21° C) and counted in 10 ml of a toluene based scintillation fluid (4 mg PPO + 50 mg POPOP per l toluene). Specific binding was calculated by subtracting non-specifically bound testosterone (^3H -testosterone + fifty-fold excess testosterone) from total bound testosterone (^3H -testosterone). Free ^3H -testosterone was measured directly from the incubation medium after centrifugation to remove the cells. Protein was determined by the method of Lowry⁵. Δ , Total; \diamond , specific; \circ , non-specific. b, Binding analysis was performed by the method of Scatchard⁹ and only specifically bound was plotted.



serum with 100 units ml^{-1} penicillin; 100 $\mu\text{g ml}^{-1}$ streptomycin, 2.5 $\mu\text{g ml}^{-1}$ fungizone (Gibco) and 1×10^{-9} M testosterone (Sigma). On reimplantation of the cells from culture into non-syngeneic Syrian hamsters, a new tumour developed and grew at essentially the same rate as the original. Since the tumour might consist of a heterogeneous cell population with a small number of cells responding to testosterone, a second trypsinisation was performed and the resulting cells were cloned in the presence of 1×10^{-9} M testosterone using Falcon microtest plates³. Cells from each clone were frozen in growth medium with 10% DMSO and saved for later analysis. Of the clones analysed to date, one designated DDT₁, has been shown to have a high steroid binding activity. The DDT₁ cell line shows several characteristics of a permanent cell line such as log-phase growth in suspension culture and formation of tumours on reimplantation into animals.

Several experiments have now shown that the DDT₁ cells respond to a physiological dose of testosterone or DHT by

FIG. 2 a, Sucrose gradient analysis was performed in linear 5–20% sucrose made up in 10% glycerol containing 20 mM Tris, 100 mM KCl, and 10^{-6} M phenyl methyl sulphonyl fluoride (PMSF) at pH 7.45. Gradients (4.8 ml) were prepared with a Buchler University Gradient Former and 0.2 ml fractions of 150,000g cytosol containing 2.3 mg protein were layered just before centrifugation at 45,000 r.p.m. for 17 h at 2° C in the Beckman SW 50.1 head. Five drop fractions were collected and counted at 25% efficiency in 13 ml of scintillation fluid (1 l of toluene + 4 gm PPO + 50 mg POPOP diluted to 1.3 l with absolute ethanol). Cytosol was prepared by homogenising the cells with 15 strokes of a dounce type B in 20 mM Tris, 2 mM MgCl_2 , 2 mM CaCl_2 , 0.5 mM mercaptoethanol, 1 μM PMSF, and 10% glycerol pH 7.45 (buffer A) at 2° C. The homogenate was centrifuged at 150,000g (Ave) in a Beckman type 65 head for 45 min at 2° C. 1, 2, 6, 7 ^3H -testosterone (Specific activity, 91 Ci mmol^{-1} , New England Nuclear) was added to buffer A to a concentration of 1.9×10^{-8} M and enough cytosol was added to dilute this to 1.9×10^{-9} M. Unlabelled steroids (testosterone or E_2) were added at a fifty-fold concentration (1×10^{-7} M) before addition of the cytosol. All the samples were incubated at least 2 h before centrifugation, (.....), ^3H -testosterone; (-----), ^3H -testosterone + testosterone; (—), ^3H -testosterone + E_2 . Sedimentation values were calculated by the method of Martin and Ames⁷ using bovine serum albumin (4.6S) and γ -globulin (7.0S) as standards. b, Whole cells were incubated for 1 h at 37° C in Dulbecco's Modified Eagle's medium (DME) with 25 mM HEPES buffer containing 6×10^{-9} M 1, 2, 6, 7 ^3H -testosterone (○—○) or 6×10^{-9} M 1, 2, 6, 7 ^3H -testosterone + 6×10^{-7} M testosterone (○—○). The cells were then washed twice in 3 ml Tris-saline pH 7.45 (all further operations at 2° C) in 12 ml conical centrifuge tubes and always centrifuged 5 min at 700g. The cells were washed a third time in buffer B (buffer A with 0.25 M sucrose instead of glycerol) and pelleted at 1,400g for 5 min. The cell pellet was transferred to a dounce type B and an equal volume of buffer B was added before homogenisation by 15 strokes. The resulting homogenate was centrifuged at 1,400g for 15 min. The nuclear pellet was resuspended by gentle vortexing and washed twice with 2 ml of buffer B. The nuclei were then diluted with an equal volume of buffer C (buffer A with 800 mM KCl) and extracted over 2 h with occasional gentle vortexing. The nuclear extract was centrifuged at 2,500g for 10 min and 0.2 ml of the supernatant containing 2.0 mg soluble protein was analysed on sucrose gradients (with the exceptions that the gradients contained 400 mM KCl) and then counted as described in (a).

increasing the rate of synthesis of DNA, as measured by incorporation of ^3H -methylthymidine (^3H -Tdr) into the hot perchloric acid soluble fraction of the cell (Table 1).

Since testosterone seems to stimulate the growth of the DDT₁ cells, we examined ^3H -testosterone retention in an intact cell system (Fig. 1). These studies indicate the presence of a high affinity receptor ($K_d = 8.5 \times 10^{-10}$ M) which is saturable at 3.6×10^{-14} M per mg protein. There are approximately 3,400 binding sites per cell.

Having demonstrated a high affinity, saturable retention of ^3H -testosterone with whole cells, we analysed this finding in a cell-free extract. Sucrose gradient analysis of 150,000g cytoplasm from the DDT₁ cells has repeatedly demonstrated a receptor binding ^3H -testosterone or its metabolites that sediments at 5.6–5.8s in 5–20% sucrose gradients made up in 10% glycerol as described in Fig. 2a. This binding is completely abolished when unlabelled testosterone is included in the incubation mixture. E_2 reduces the binding by 33.5%. Enzymatic degradation of the material in this fraction by trypsin (500 $\mu\text{g ml}^{-1}$) at 2° C for 2 h, but not RNase (500 $\mu\text{g ml}^{-1}$) or DNase (500 $\mu\text{g ml}^{-1}$), suggests that the receptor is a protein.

When whole cells are incubated 1 h in the presence of ^3H -testosterone, a receptor can be extracted from the nuclear pellet using a high salt buffer (Fig. 2b). The receptor sediments at approximately 5.1S and when unlabelled testosterone is added to the incubation media the 5.1S peak disappears.

The DDT₁ cell line seems to be the first cloned cell line that exhibits a growth response to testosterone and DHT

TABLE 1 Effect of DHT and testosterone on the rate of DNA synthesis in cell culture

	Mean μg DNA per plate* at day 4	P	Mean d.p.m. ^3H -Tdr per μg DNA*	P
Control	31.92 \pm 2.78		384.4 \pm 16.4	
8.9×10^{-9} M 5α DHT	40.88 \pm 0.99	<0.001	525.7 \pm 27.6	<0.001
8.9×10^{-9} M Testosterone	37.72 \pm 1.67	<0.005	466.5 \pm 9.6	<0.001

* Values \pm s.d.

At the time the androgens were added there were 9.7 ± 0.4 μg DNA per plate.

Replicate cultures were plated at a density of 5.75×10^5 cells per 60 mm tissue culture dish in Ham's F-10 (all media and chemicals from Gibco unless otherwise stated) with 1% penicillin-streptomycin solution, 1% fungizone, and 10% foetal bovine serum. The serum had been heated for 1 h at 55°C in the presence of 3% by weight activated charcoal (G-60, Matheson Scientific) and filter sterilised. Steroids were not added for 24 h to avoid any effect the androgens might have on plating efficiency. On day 2 all cultures were changed and androgens were added as designated. The control group received 0.01% carrier ethanol but no added steroids. The androgen-treated cultures received the same medium but with the addition of 8.9×10^{-9} M testosterone or DHT. Thereafter, the culture media were changed every 24 h for 4 d. Any cells floating in the expended media were retrieved by centrifugation and saved for inclusion in the final DNA totals. ^3H -methyl thymidine (^3H -Tdr) (NET-027Z Specific activity 43 Ci mmol $^{-1}$) was added on day 4 directly to the cultures in 0.05 ml of Hanks' balanced salt solution to a final concentration of 0.4 $\mu\text{Ci ml}^{-1}$. After incubation for 2 h the cells were collected by scraping, and washed twice with ice-cold Tris-saline pH 7.4. All centrifugations were at 800g. The cold acid-soluble counts were removed with three 3 ml 10% TCA washes at 0°C . The cells were then incubated at 70°C for 30 min in 0.5 N PCA. After centrifugation a 0.2 ml aliquot was counted for tritium as described in Fig. 2a. DNA analysis was performed by the method of Burton⁶. Probability was calculated using Student's *t* test of means. In all groups five samples were used.

which also contains cytoplasmic and nuclear androgen receptors^{10,11} although Smith *et al.*¹² have described androgen-responsive mammary tumour cells which bind DHT. The cytoplasmic protein in some respects resembles androgen binding protein from other cells but is different from the binding protein found in sera^{4,8}. Furthermore, the steroid binding protein in the DDT₁ cells seems to be involved in the nuclear translocation of testosterone or its metabolites to a nuclear acceptor. Therefore, we conclude that the DDT₁ cell line will prove a useful tool in the search for the mechanism of action of androgens.

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Serotonin and pituitary-adrenal function

SEVERAL reports have indicated a relationship between the pituitary-adrenal secretion of steroids and brain serotonin (5-hydroxytryptamine) in the rat^{1,2}. Serotonin is believed to be a transmitter or regulator of neuronal function. We have previously investigated the effects of altering brain serotonin concentrations on the daily fluctuation of the pituitary-adrenal system³. We have investigated this problem further, by evaluating the response of the pituitary-adrenal system to a stress stimulus in the rat. Our approach was to either inhibit brain serotonin synthesis with *p*-chlorophenylalanine (PCPA), or to raise the concentration of serotonin with precursors such as tryptophan or 5-hydroxytryptophan (5-HTP)⁴.

We used male Sprague-Dawley rats (150–200 g). They were kept in a 12L:12D environment (lights on at 0700 h) and given food and water *ad libitum*. Serum corticosterone was assayed fluorometrically, while the determination of serum ACTH was made by bioassay⁵. The concentrations of brain serotonin were determined after isolation, using a weak cation exchange resin and fluorometric assay⁶. One hundred and eighty rats divided into two groups were injected intraperitoneally (i.p.) with pyrogen-free saline or PCPA (Pfizer) in saline at a dose of 300 mg kg $^{-1}$ d $^{-1}$.

Daily injections of PCPA for 4 d greatly enhanced and sustained the peak ACTH response to ether stress ($P < 0.05$) (Fig. 1). To assess whether this enhanced response was due to an effect of PCPA on the diurnal variation in

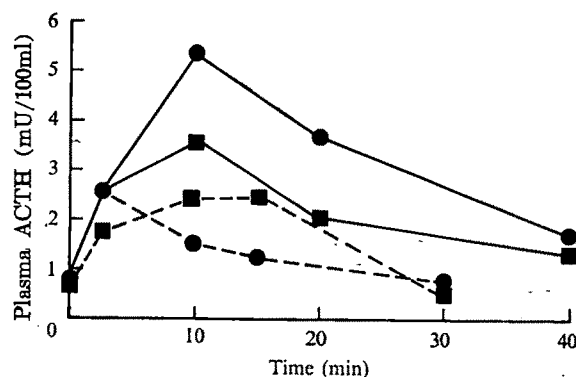


FIG. 1 Time course of the ACTH response to 1 min of ether stress. Each value represents the mean of eight animals. ●, Morning; ■, evening; —, PCPA; ---, saline.

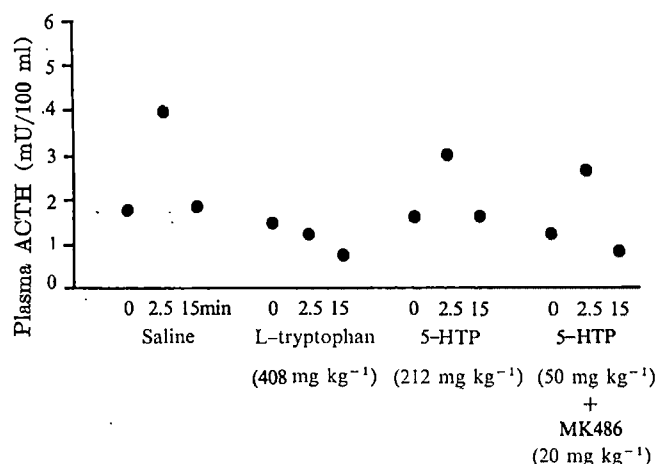


Fig. 2 Effect of serotonin precursors on the ACTH stress response. L-Tryptophan (408 mg kg^{-1}) or 5-HTP alone (212 mg kg^{-1}) or 5-HTP (50 mg kg^{-1}) with the peripheral decarboxylase inhibitor, L- α -hydrazinomethyl-dihydroxyphenylalanine (MK 486; 200 mg kg^{-1}) were injected i.p. into groups of rats (eight per group). The animals were adrenalectomised 24 h before the study to enable us to study the ACTH response isolated from the feedback effects of circulating steroids. The rats were studied 2 h after injection of the serotonin precursors when brain serotonin levels have been reported at maximal levels after the injection of precursors¹¹ and when pituitary adrenal activity has usually returned to normal in control animals after the stress of an injection. Animals were either decapitated and bled at zero time without ether stress or decapitated and bled 2.5 or 15 min after the stress of ether.

the rate of secretion of ACTH in response to stress, the time course of this response to 1 min of ether stress was determined at 0800 h or 2000 h. Figure 1 shows that, whereas in the controls, blood ACTH concentrations after stress peaked at 2.5 min in the morning and at about 15 min in the evening, depletion of serotonin not only enhanced the response, but also greatly altered the rhythm in the response to ether stress; maximum stress response occurred at 10 min, both in the morning and in the evening. Thus, inhibition of brain serotonin synthesis enhanced the stress response, in addition to altering the pattern of the pituitary-adrenal stress response. This agrees with our previous findings that daily injections of PCPA for 2–4 d raised the morning low, and prevented the evening rise in plasma corticosterone in unstressed animals^{1,3}.

Our alternative approach to the study of the role of brain serotonin in the regulation of pituitary-adrenal function was to attempt to raise brain serotonin by the use of the precursors tryptophan (J. T. Baker Co) and 5-HTP (Calbiochem). Tryptophan is the dietary amino acid which is converted to 5-HTP, the immediate precursor of serotonin. We also used a decarboxylase inhibitor (MK-486, Merck, Sharpe and Dohme), which only acts peripherally in combination with 5-HTP to increase brain serotonin levels by inhibiting serotonin formation outside the brain. Adrenalectomised animals (eight per group) were stressed with ether 2 h after the administration of serotonin precursors, and the pattern of the rise in ACTH was studied (Fig. 2). In these adrenalectomised animals, tryptophan increased brain serotonin content by an average of 33%, and significantly reduced the stress-induced secretion of ACTH ($P < 0.01$) which, in the saline controls, occurred by 2.5 min, and returned to prestress levels by 15 min. 5-Hydroxytryptophan alone, or in combination with MK-486, increased brain serotonin content by 2.4-fold and 1.2-fold respectively, and reduced the stress response in these animals significantly ($P < 0.05$). Thus, these precursors of serotonin tended to decrease or inhibit the pituitary-adrenal response to stress. It is interesting that tryptophan which produced an

increase of about 33% in brain serotonin had a greater effect on ACTH than 5-HTP, which produced a much greater increase. This is consistent with the conclusion of Moir and Eccleston that tryptophan loading is the more physiological method of increasing brain serotonin content⁷.

Preliminary reports on human studies also appear to support this serotonin pituitary-adrenal interrelationship. 5-Hydroxytryptophan seems to reduce the 24 h excretion of 17-hydroxycorticosteroids in human volunteers^{3,8}.

It is now reasonably well accepted that the pituitary-adrenal system functions as a closed loop. In this system, the positive vector or 'driving force' corresponds to environmental stimuli, acting through hypothalamic neuro-humoral pathways⁹ and the negative vector corresponds to the concentration of corticosteroids which act by feedback inhibition on the pituitary-adrenal system¹⁰. We found that the stress response was enhanced by inhibition of serotonin synthesis, and found indications of a reduction of the stress response by serotonin precursors. This suggests that serotonin may mediate or modulate this negative feedback mechanism regulating pituitary-adrenal function.

If this hypothesis were correct, then the inhibitory action of corticosteroids on steroid output would be expected to be less effective in animals in which brain serotonin was depleted. Figure 3 illustrates the results of an experiment testing this possibility, and compares the corticosterone stress response inhibiting properties of two doses of a steroid (prednisolone) in control rats and in animals whose brain serotonin content was markedly reduced by pretreatment with PCPA. Both doses of the steroid effectively inhibited the response to the stress of ether and laparotomy in the control animals. In serotonin-depleted animals, neither dose was capable of blocking the stress response, further indicating that serotonin is indeed involved in the negative feedback to the pituitary-adrenal system.

These results, taken together, lead us to speculate that in some disease states, such as Cushing's syndrome, where there is a similar inability of corticosteroid to suppress the pituitary-adrenal system, there is a defect in serotonergic neuronal processes that impairs pituitary-adrenal feedback mechanisms. Thus, we reason that serotonin precursors may be useful as a diagnostic screening test to identify cases of Cushing's syndrome with the postulated serotonergic defect. Further, serotonin precursors (tryptophan or 5-HTP, with

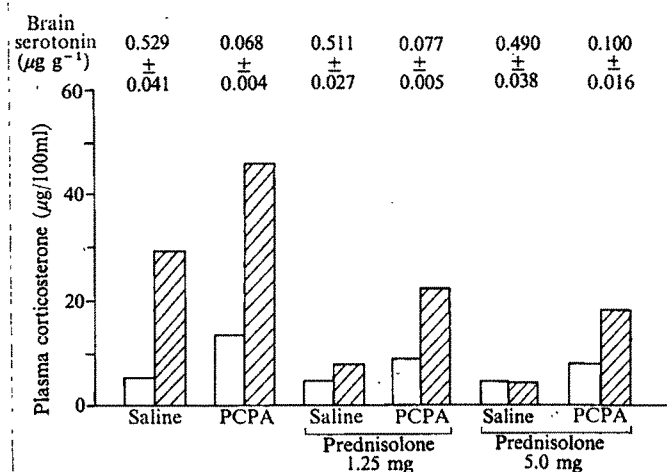


Fig. 3 The effect of serotonin depletion on the inhibition of corticosterone release by prednisolone. Groups of rats (eight per group) were stressed with ether and laparotomy 24 h after two daily injections of PCPA ($300 \text{ mg kg}^{-1} \text{ d}^{-1}$ i.p.) or saline and 4 h after the injection of the steroid (1.25 mg or 5.0 mg per 100 g body weight subcutaneously). Brain serotonin concentrations were determined on the unstressed group of rats. Open columns, unstressed; hatched columns, 15 min after ether laparotomy stress.

or without a peripheral decarboxylase inhibitor), or agents which stimulate serotonin receptors, may be useful in the therapy of some forms of Cushing's disease.

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Unresponsiveness of human B lymphocytes to phytohaemagglutinin

A WIDELY accepted, but unproven, distinction between human T (thymus-derived) and B (non-thymus-derived) lymphocytes is their differential response to mitogenic stimulation with phytohaemagglutinin (PHA). It has been claimed that such a distinction does not truly exist and that the human B cell, unlike the murine B cell, transforms in the presence of PHA¹. An editorial² commenting on the consequences of this claim appropriately emphasised the danger of extrapolating data from one species to another. This communication presents evidence that pure populations of human B cells do not transform in response to stimulation with PHA. It also stresses the need of reducing the contamination of B cell preparations by T cells when studying the *in vitro* behaviour of the human B lymphocytes.

Pure populations of human T and B lymphocytes were obtained from tonsils using a two-step fractionation procedure. Cells were first fractionated on discontinuous gradients of bovine serum albumin (BSA)³ and then separated on the basis of their reactivity with the sheep red cell intermediates E and EAC1423 (EAC3) (Fig. 1).

Tonsil cells sedimenting in fraction 4 of the BSA gradient (interface of 23 and 25% BSA) were enriched in T cells. Forty to fifty per cent of the cells in fraction 4 formed rosettes with E (Fig. 1b), and only 15–20% reacted with EAC3 and stained with fluorescent antisera to human immunoglobulins (Fig. 1c, d). Cells sedimenting in fraction 7 of the BSA gradient (interface of 29 and 31% BSA) were almost exclusively B cells. Ninety to ninety-five per cent of

the cells in this fraction reacted with both EAC3 and fluorescent antisera to human immunoglobulins (Fig. 1c, d), and fewer than 5% formed rosettes with E (Fig. 1b).

B cells were obtained by taking the E non-rosette-forming cells from fraction 7. Ninety-eight to one hundred per cent of these cells exhibited reactivity with EAC3 and surface staining with fluorescent antisera to human immunoglobulins. Less than 2% of these cells formed rosettes with E on retesting (Table 1). T cells were obtained by taking either the

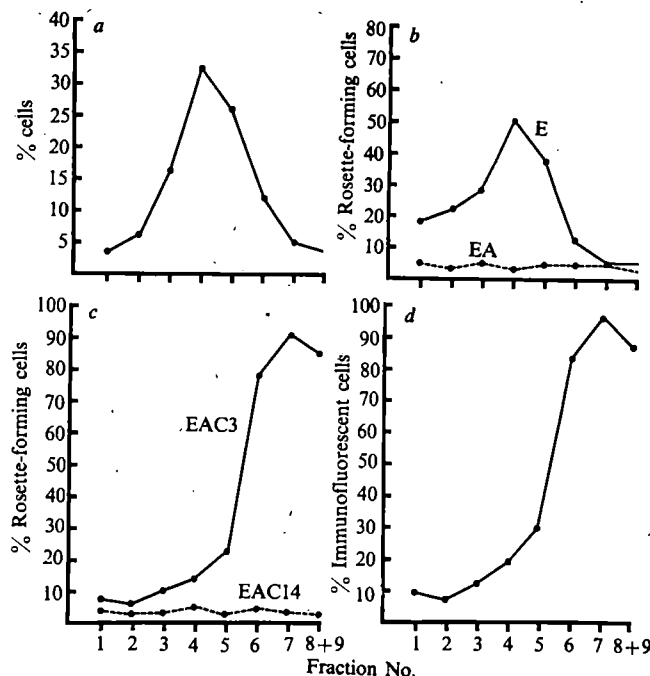


FIG. 1 *In vitro* response of tonsil lymphocytes separated into nine fractions by centrifugation in a discontinuous gradient of 17–35% BSA. a, Relative distribution of lymphocytes after purification by passage through glass beads. b, Rosette formation with sheep erythrocytes (E) compared to EA as a control. c, Rosette formation with EAC3 compared to EAC14 (control). d, Percentage of cells staining with antisera to human immunoglobulins. Values represent the average of four experiments. Human tonsils were teased in Medium 199 (Microbiological Associates), and the resulting suspensions were passed through columns of glass beads at 37° C to remove adherent cells. Column eluates contained more than 90% viable lymphocytes as determined by trypan blue exclusion. Cells in the eluate were fractionated on discontinuous gradients of BSA by a modification of the method of Dicke *et al.*³. Cells from each fraction were washed in Medium 199, then tested for their ability to form rosettes with the sheep red cell intermediates E and EAC1423, as well as for surface staining with fluorescent antisera to human immunoglobulins. Red cell intermediates E, EA, and EAC14 were prepared using human components of complement and were suspended at a concentration of 1% in phosphate-buffered saline (PBS). Lymphocyte suspensions were adjusted at 2×10^6 cells ml⁻¹ in PBS containing 10% serum from a donor with type AB Rh⁺ erythrocytes and absorbed three times with sheep erythrocytes. Equal volumes of lymphocyte and E or E-intermediate suspensions were mixed. Tubes containing E and EA were incubated for 30 min at room temperature, while those containing EAC3 and EAC14 were incubated for the same period of time at 37° C. Following this initial incubation, all tubes were centrifuged at 200g for 4 min at 4° C, and the pellets were incubated at 4° C for 1 h. Cells were then resuspended and examined microscopically, and the percent of rosette-forming cells determined. Lymphocytes intimately associated with four or more red cells were considered to have formed rosettes. Immunofluorescence was performed using fluorescein-conjugated antisera raised in goats against human IgG, IgM, and IgA myeloma proteins. Each antiserum was rendered monospecific for the heavy chain of a single class of immunoglobulins by absorption on suitable antigens coupled to Sepharose 4B by cyanogen bromide.

E rosette-forming cells or the EAC3 non-rosette forming cells from fraction 4. T cell preparations contained less than 5% EAC3-reactive immunofluorescent staining cells (Table 1).

B cells uniformly failed to transform in response to stimulation with PHA; stimulation index (s.i.) = 2 (Table 2). PHA caused vigorous proliferation of T cells with s.i. ranging from 57 to 166 (Table 2). The unresponsiveness of the B cell to PHA could not have been due to cell death. Viability of B cells after 72 h in culture always exceeded 65% as determined by trypan blue exclusion, and parallel B cell cultures always transformed normally in response to pokeweed mitogen (PWM) added at a concentration of 1/100 (s.i. 4-11).

Lack of adherent cells was ruled out as a cause for the unresponsiveness of the B cell cultures to PHA. Adherent cells, T cells, and B cells were prepared from the same tonsil. In combination experiments, 1×10^5 to 2×10^5 adherent cells were added to 1×10^6 B or T cells. The results in Table 2 show that the addition of adherent cells did not affect the response of the B cell to PHA, but increased the mitogenic response of the T cell to PHA. Adherent cells by themselves did not transform in response to PHA.

The effect of the contamination of purified B cell cultures by T cells on the PHA response was studied. Five per cent (5×10^5) T cells were mixed with 95% (95×10^5) B cells from the same donor. The overall response of the mixture to PHA was found to exceed by far the sum of the responses of the individual T and B cell components (Table 2). This effect was not seen when either one of the two components was previously subjected to irradiation with 7,000 r.

An attempt was made to determine if this enhancing effect was due to the action of a soluble mediator released by T cells and acting on B cells. The results in Table 3 show that supernatants from resting T cells, as well as supernatants from PHA-activated T cells, failed to cause transformation in B cells. T cells proliferated more vigorously in the presence of the PHA-containing supernatants than in the presence of PHA alone (Table 3).

That human B cells fail to transform with PHA was also shown by studying lymphocytes from a 6-month-old boy with the diagnosis of severe combined immunodeficiency. These lymphocytes exhibited characteristics of B cells; 3% formed rosettes with E; 95% showed immunofluorescence with an anti- μ -chain antiserum. Stimulation indices of 1.9 and 2.0 with PHA and 7.5 and 9.1 with PWM were obtained when these cells were cultivated *in vitro*.

The data presented here regarding the human B cell response to PHA are in agreement with those on B cells from

TABLE 2 Proliferative response of human B, T and adherent cells to PHA

Cells in culture	³ H-Thymidine incorporated per culture		s.i.
	Without PHA c.p.m.	With PHA c.p.m.	
1×10^6 B cells	1,011	1,332	1.3
1×10^6 T cells	864	96,722	112.0
1×10^6 adh cells	1,235	967	0.8
1×10^6 B cells + 1×10^5 adh cells	1,174	1,305	1.1
1×10^6 T cells + 1×10^5 adh cells	908	117,460	129.0
5×10^5 T cells	385	17,033	44.2
95×10^5 B cells	1,158	1,388	1.2
5×10^5 T cells + 95×10^5 B cells	1,147	38,565	33.6

Lymphocytes were cultured at a final concentration of 1×10^6 cells ml^{-1} in Medium RPMI-1640 (Grand Island Biological Company) supplemented with 15% serum obtained from an individual with AB Rh⁺ erythrocytes and containing penicillin ($50 \mu\text{g ml}^{-1}$), streptomycin ($50 \mu\text{g ml}^{-1}$), kanamycin ($50 \mu\text{g ml}^{-1}$), and mycostatin ($50 \mu\text{g ml}^{-1}$). Duplicate 1 ml cultures were set up in 12×75 mm Falcon plastic tubes and incubated in a humidified atmosphere of 5% CO_2 in air. Phytohaemagglutinin (PHA-P, Difco) was added at a final concentration of 1:100. Seventy-two hours after the onset of incubation, cultures were pulsed with ³H-thymidine (1.9 Ci mmol^{-1} , $1 \mu\text{Ci ml}^{-1}$; New England Nuclear). Sixteen hours later, cultures were collected and the amount of ³H-thymidine incorporated into DNA was determined by the filter paper disk technique³. Results were expressed as c.p.m. incorporated per culture and as stimulation indices (s.i.) where

$$\text{s.i.} = \frac{(\text{c.p.m. of } ^3\text{H-thymidine incorporated by the stimulated culture})}{(\text{c.p.m. of } ^3\text{H-thymidine incorporated by the unstimulated culture})}$$

Adherent (adh) cells from tonsils were prepared by fractionating aliquots of single cell suspensions on BSA gradients without previous passage over glass beads. Fraction 3 of such gradients was enriched in monocytes (30-40%), determined by their ability to stain with neutral red. Cells from fraction 3 were reacted with E, after which the E non-rosette-forming cells were separated over a Ficoll gradient, washed, and resuspended at a concentration of $3 \times 10^7 \text{ ml}^{-1}$ in Medium 199, supplemented with 30% AB Rh⁺ human serum. Five millilitre aliquots of this cell suspension were added to 100 mm Petri dishes. Dishes were incubated at 37°C for 30 min, rocked gently for 5 min, and washed with 10 ml of M199. The rocking and washing step was repeated five times. Cells remaining adherent to the bottom of the dish at the end of the washing procedure were collected with a rubber policeman, washed, and resuspended in culture medium at a concentration of 1×10^6 cells ml^{-1} . Eighty to ninety per cent of the cells in this adherent cell preparation stained with neutral red. Identical results to those shown in the Table were obtained in four different experiments.

other species like the mouse, guinea pig, and chicken⁴⁻⁷. Our data, however, are in conflict with those of Phillips and Roitt¹, who used human B cell preparations in which at least 10% of the cells were non-B cells.

It seems that in man, as in the mouse, though pure B cells do not transform with PHA, a mixture of 5% T cells and 95% B cells exhibits a strong proliferative response to PHA which exceeds the sum of the responses of the individual T and B cell components⁸. In the mouse, karyotypic analyses of such mixtures revealed that by day 2 of culture 35% of the observed mitoses were B cells and that by day 3 of culture 97% of the mitoses were B cells. Similar data are not available for human cells.

The more-than-additive PHA response of human T and B cell mixtures could not be attributed to a soluble mediator released by T cells and acting on B cells. Human B lymphocytes, like chicken B lymphocytes, failed to proliferate in the presence of supernatants obtained from PHA-activated T cells⁷. But, in the case of *in vitro* stimulation with antigen to which cell donors are immune, it was found that the human B cell does not transform with antigen alone but transforms

TABLE 1 Characteristics of B and T lymphocyte preparations from human tonsils

Cell Source	T Lymphocytes*	B Lymphocytes†
E (+)	>95%	< 2%
EAC3 (+)	< 5%	>98-99%
Immunofluorescent (+)	< 5%	>98-100%

* E-reactive cells from fraction 4 of the BSA gradient

† E non-reactive cells from fraction 7 of the BSA gradient

Cells from fractions 4 and 7 of the BSA gradient were reacted with E or EAC3, and the lymphocyte sheep red cell mixtures were layered on top of Ficoll-Hypaque gradients and centrifuged at 400 g and 18°C for 45 min. Non-rosette-forming cells collected at the interface of the gradient, while rosette-forming cells and red cells collected at the bottom of the gradient. Cells collected from the bottom of the gradient were treated for 10 min with 0.87% NH_4Cl to lyse the red cells. Lymphocytes collected from the Ficoll gradient were washed three times in Medium 199 before culture. Cell recovery, following Ficoll gradient separation, averaged 70-80%. Viability of the recovered cells always exceeded 90%.

TABLE 3 Effect of T cell supernatants on proliferative response of human B and T cells to PHA

Addition to culture	³ H-Thymidine incorporated per culture of	
	B cells	T cells
None	c.p.m. 925	c.p.m. 812
PHA	1,067 (1.1)	82,638 (101)
24-h supernatant from T cells	877 (0.9)	763 (0.9)
24-h supernatant from T cells + PHA	1,168 (1.3)	78,314 (96.4)
24-h supernatant from PHA-activated T cells	1,309 (1.4)	98,561 (120)
48-h supernatant from PHA-activated T cells	993 (1.1)	91,426 (113)

Supernatants were derived from cultures of T cells incubated at 1×10^7 cells ml⁻¹ with or without PHA. Culture supernatants were filtered through 0.45 μ m filters (Millipore Corporation, Bedford, Massachusetts) and stored at -20° C until tested. Supernatants were added at 1:2 dilution to both B and T cells cultures. PHA concentration was adjusted to 1/100 in all cultures. Identical results were obtained in four different experiments. Values in between parentheses represent s.i.

vigorously (s.i. 20-40) in the presence of supernatants derived from antigen-activated T cells⁹. In the process, the B cell loses its C3 receptor, accumulates rough endoplasmic reticulum, and engages in antibody secretion. If the human B cell transforms with PHA only in the presence of T cells, then this could mean that intimate B cell to T cell contact is needed. It would be interesting to know if human B cells, like their murine counterparts, possess receptors for PHA as T cells but do not respond to the mitogen¹⁰ or if they lack a receptor for PHA.

It is clear from this work that it is necessary when studying the behaviour of the human B lymphocytes to reduce as much as possible contamination of the purified B cell preparations with T cells. Caution should also be exercised in extrapolating data from mouse to man, as the B cells from the two species have been recently shown to behave differently *in vitro* in response to stimulation with phytohemagglutinins, antigens, and lipopolysaccharides (R.S.G. and E.M., unpublished).

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Independent behaviour of blood group A- and B-like activities in gastric carcinomata of blood group AB individuals

SIMMONS and Perlmann¹ have provided evidence that the carcinoembryonic antigen (CEA) (ref. 2) is an incomplete blood group substance that lacks the type 1 chain in most of the material studied. This deficiency was explained by deletion or repression in gastrointestinal carcinomata of genes and related transferases required for the synthesis of the blood group specific macromolecules. The chains which are not incorporated apparently accumulate as suggested by the work of Hakomori *et al.*³. Since A-, B-, H-, and Le^a-specific sequences in the type 2 chain were also missing in the CEA preparations¹ Simmons and Perlmann concluded that gastrointestinal adenocarcinomata may, in addition, have more specific defects localised at the ABO, H, and Le loci. Morphological studies dealing with the distribution of blood group-like antigens in carcinomata⁴⁻⁹ have provided further proof for the disturbance of the synthesis of blood group substance in neoplasia. Davidsohn⁶ reported a correlation between the histological degree of differentiation of the tumour and the presence of blood group substance and suggested an inverse relationship between the ability of a tumour to produce blood group substance and to metastasise. In the course of morphological studies dealing with the relationship between blood group substance and CEA in tumour tissue, we studied five specimens of surgically removed gastric carcinomata from patients with the blood group A₁B and we observed independent behaviour of the A and the B specificity in four of them in certain areas of the malignant growth.

The specimens were fixed in neutral buffered formalin, embedded in paraffin and subsequently treated for the demonstration of blood group antigens by the modified mixed cell agglutination reaction (MCAR)¹⁰ as described by Davidsohn (for review see ref. 6). Commercial antisera to blood group substance (BG)-A and -B (Ortho) were concentrated to a haemagglutination titre of approximately 1:500.

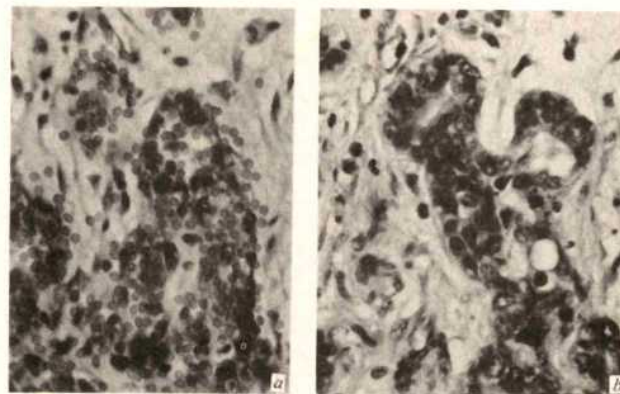


FIG. 1 Moderately differentiated gastric adenocarcinoma. a, Positive MCAR (BG-B); b, negative MCAR (BG-A). Serial sections. $\times 285$, Haematoxylin and eosin (HE).

TABLE 1 A and B blood group substance—like activity in gastric cancer tissue of patients with blood group AB

No.	11027	14283	1899	1896	2878
Histology					
Adenocarcinoma	—	A neg/B neg	A neg/B neg A neg/B neg	A neg/B pos A pos/B pos A neg/B neg	A pos/B neg
Globocellular carcinoma (anaplastic)	A pos/B pos A neg/B pos	A neg/B pos A neg/B neg	—	—	A pos/B pos
Signet ring cell carcinoma	—	—	—	A neg/B neg	A neg/B neg

Ulex europaeus extracts were used to detect H activity. According to ref. 6 blood group substances soluble in both water and alcohol are preserved during formalin fixation and paraffin embedding. After completion of the specific red cell adherence reaction the specimens were fixed in 1–5% glutaraldehyde and stained with haematoxylin-eosin. Histologically the tumours showed a variety of patterns from rather well-differentiated adenocarcinoma to anaplastic globocellular growth. Signet ring cells were present in two carcinomas. The normal superficial as well as the intestinal metaplastic mucosa were rich in both BG-A and BG-B in all of our five cases in a distribution typical for secretors^{11,12}. The endothelial cells of the blood vessels and the erythrocytes in the normal tissue as well as within the carcinoma were positive with respect to BG-A and BG-B in every case thus providing appropriate positive controls. Reagent controls were non-isologous antiserum and isologous indicator erythrocytes and *vice versa*. No relationship between the presence of blood group substances and the histological degree of differentiation was observed in our cases and different distribution patterns of BG-A and -B activities were observed even in morphologically identical areas (Table 1). No H activity was demonstrable in the tumours although control specimens of blood group O and A₂ gave positive results with the *Ulex* extract used.

CEA was postulated to result from defective synthesis of blood group substances in gastrointestinal carcinomata

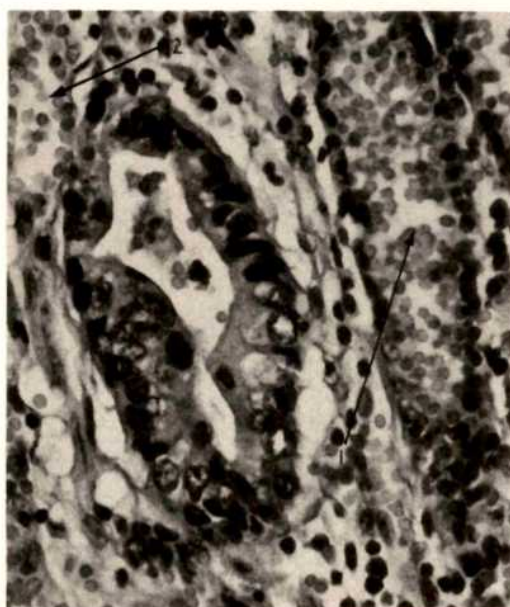


FIG. 2 Moderately differentiated gastric adenocarcinoma (same tumour as in Fig. 1). Negative MCAR for BG-B (shown) as well as for BG-A (not shown) in the tumour. Positive reaction in adjacent normal mucosa (1) and vessel (2). $\times 400$, HE.

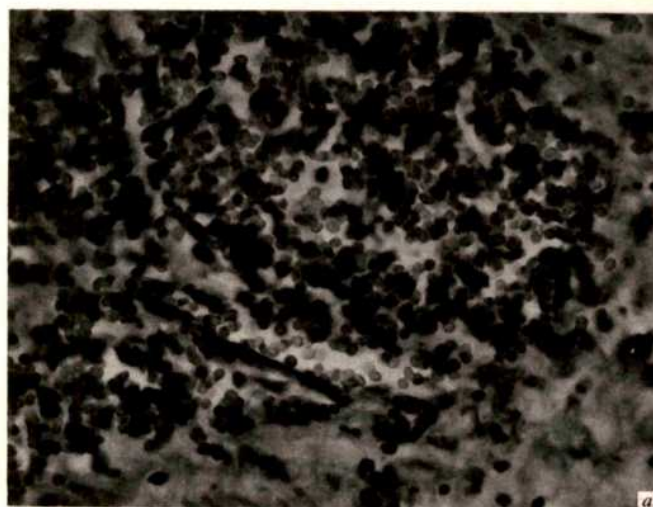


FIG. 3 Anaplastic globocellular carcinoma. a, Positive MCAR (BG-B); b, negative MCAR (BG-A). Blood group substance-positive endothelial cells (1). Serial sections. $\times 350$ HE.

due to genetic defects¹. Our results allow us to localise possible genetic defects more precisely. α -N-acetyl-D-galactosaminyl transferase and α -D-galactosyl transferase, respectively, that attach the appropriate sugars in 1 \rightarrow 3 linkage to the β -galactosyl unit in an H-active structure depend upon intact A and B genes, respectively¹³. Since these transferases have identical acceptor substrate requirements¹³ the selective loss of either BG-A or BG-B is most likely to be due to deletion or repression of the corresponding gene (either A or B) in the ABO locus. A similar mechanism was discussed to explain the appearance of A₂, A₂B, B and O erythrocytes in a patient with leukaemia¹⁴. Disappearance

of both A and B blood group substances in other parts of the same carcinoma confirms that the genetic and related enzyme defects vary even in the same tumour. The exact relationship between blood group substances and CEA in cancer tissue is not yet established. Different degrees of genetically determined lesions are conceivable: selective loss of one antigenic specificity may represent the minimal change in a process that leads to the development of a CEA-specific chemical structure.

Other, less likely explanations of the selective loss of one BG specificity in A,B patients should also be considered. One is the absence of substrates such as N-acetyl-D-galactosamine or D-galactose in the tumour. This possibility is unlikely since the intact precursor molecule relies on the presence of these carbohydrates. It is also possible that a specific A- or B-destroying enzyme exists in the tumour cells.

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Model for the binding of multivalent antigen to cells

It has been suggested frequently¹⁻⁴ that antigen must be presented to immunocompetent cells in multivalent or aggregated form in order to stimulate the cells to proliferate and secrete antibodies or otherwise respond. Multivalent antigens, that is antigen molecules with more than one identical epitope per molecule, seem to bind multivalently and irreversibly to the antibody-like receptors on immunocompetent cells^{5,6}, and T cells or their products may serve to aggregate antigen molecules so as to present them to other cells in multivalent forms⁷. The binding of multivalent ligands to lymphocyte receptors is known to induce receptor-ligand lattice formation and in many cases to induce mitosis and antibody secretion from B cells^{4,8,9}.

It is also known that, after exposure of an animal to antigen, the affinity of the antibodies which are produced will often increase with time^{10,11}. This maturation of the immune response could be understood^{12,13} easily if the binding of antigen to cells and cell proliferation were governed by the equilibrium constant for receptor (antibody)-epitope

binding. A small (and decreasing) concentration of free antigen would then bind cells with high (and increasing) affinity receptors and would selectively stimulate such high affinity cells. If, however, the antigen-to-cell binding is actually multivalent and irreversible⁶, it is not clear how it can be determined by the receptor-epitope equilibrium constant, and consequently how the maturation can be induced.

The purpose of this communication is to present a simple model of how the rate of multivalent and irreversible binding can be governed by an equilibrium constant for epitope-receptor binding. Although the model is highly simplified, it suggests several testable relationships between important measurable quantities.

The model is depicted in Fig. 1. Consider a mixture of multivalent antigen molecules and cells to which they can bind. In binding to a cell, an antigen molecule is assumed first to bind to a single receptor at one binding site with rate constant k_a . From this configuration the antigen molecule can either dissociate with rate constant k_d or form a bond with another receptor molecule, with rate constant k_i , thereby establishing multivalent and irreversible binding. We ignore, or alternatively, include in k_d ¹⁴, the binding of one antigen molecule to two sites on the same receptor molecule.

Let c be the concentration of free antigen, ρ and ρ_0 be the concentrations of free and total receptor sites, m be the concentration of singly bound antigen molecules, and M be the concentration of multiply bound antigen molecules, with on the average n bonds per molecule. Then the equations of the model are

$$\rho(t) = \rho_0 - m(t) - nM(t) \quad (1)$$

$$\frac{dm}{dt} = k_a c \rho - k_d m - k_i m \rho \quad (2)$$

$$\frac{dM}{dt} = k_i m \rho \quad (3)$$

Different cells may have different values of k_a , k_d , k_i , and ρ_0 . We assume that the reversible binding is near equilibrium so that as a first approximation we set $dm/dt = 0$ in equation (2) to obtain,

$$k_a c \rho = k_d m + k_i \rho m \quad (4)$$

Two limits can be considered.

(1) If $k_d \ll k_i \rho$, the dissociation rate is slow compared with the rate of establishing multivalent binding. Then from equation (4) $k_i \rho m \approx k_a c \rho$ and substituting in equation (3)

$$\frac{dM}{dt} = k_a c \rho. \quad (5)$$

In this limit, as soon as a first bond is established subsequent bonds will be established. The rate-limiting step is first bond formation. The various antigen binding cells will differ somewhat in their values of k_a , and perhaps ρ_0 , but variation in the epitope-receptor equilibrium constant, $K \equiv k_a/k_d$, is expected^{14,15} to be primarily due to variation in k_d , not in k_a . Therefore in this limit we do not have a mechanism for preferentially binding to and selecting cells based on their equilibrium binding.

(2) If $k_d \gg k_i \rho$, the dissociation rate is rapid compared with the rate to establish multiple bonds. Then equation (4) gives $m = Kc\rho$. With equation (1) this gives

$$\rho = \frac{\rho_0 - nM}{1 + Kc}, \quad m = \frac{Kc}{1 + Kc} (\rho_0 - nM) \quad (6)$$

so that equation (4) becomes

$$\frac{dM}{dt} = k_i \frac{Kc}{(1 + Kc)^2} (\rho_0 - nM)^2. \quad (7)$$

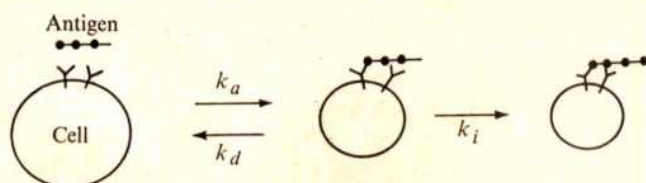


FIG. 1 Multivalent antigen is assumed first to combine reversibly with a cell receptor, with forward and reverse rate constants k_a and k_d , and then irreversible binding is established to further receptors with rate constant k_i .

In this limit, the times for both first and second bond formation are important, and the rate of establishing multivalent binding is proportional to $Kc/(1 + Kc)^2$. For cells with small values of K (that is, $K \ll 1/c$), the rate is slow because few receptors have (singly bound) antigen molecules stuck to them. If any cells have such large values of K that $K \gg 1/c$, they will also have slow rates of multivalent binding because most receptors are already singly bound to antigen molecules and therefore unavailable for establishing multiple bonds.

Equation (7) suggests a simple model for maturation of immune responses when irreversible multivalent binding of antigen is required for cell stimulation. In particular, assume that the rate of establishing multivalent binding must exceed some value, $1/T$, in order for the cells to be stimulated. Here T is a time which might be the natural lifetime of immunoglobulin receptors on cell surfaces^{16,17}. If the maximum value of the relative rate, $\rho_0^{-1} dM/dt$, must exceed $1/T$, we obtain from equation (7)

$$k_i \rho_0 \frac{Kc}{(1 + Kc)^2} > \frac{1}{T} \quad (8)$$

Only cells with K values such that the criteria of equation (8) are satisfied would be stimulated by a free antigen concentration, c . This equation is very similar to some^{12,13} which were derived for (monovalent) equilibrium binding and which gave maturation kinetics in reasonable agreement with experiment. In these studies¹³, high zone tolerance was postulated (*ad hoc*) in that cells with large values of Kc were not stimulated and were sometimes assumed to be killed. Equation (8) clearly predicts that cells with sufficiently high values of Kc will not be stimulated.

Equation (7) can be solved readily. If antigen is first introduced at $t = 0$ and c is thereafter constant, the solution is

$$M = \left[\frac{\rho_0}{n} \frac{\alpha \rho_0 t}{1 + \alpha \rho_0 t} \right] \quad (9)$$

where $\alpha \equiv nk_i Kc/(1 + Kc)^2$.

Which limit is more likely to apply? For the dissociation of haptens or small antigens from antibodies, k_d is typically¹⁵ 1–100 s⁻¹, so that we assume that for antigen bound to a receptor at one site, typically $k_d \gg 1$ s⁻¹. For those antigens that can rapidly establish a double bond to one receptor molecule, k_d is much smaller, perhaps typically¹⁴ $\sim 10^{-3}$ s⁻¹. The rate $k_i \rho_0$ to establish a bond to a second receptor molecule will depend on the diffusion of receptors in the membrane and has been estimated to be¹⁸

$$k_i \rho_0 = an t_D^{-1} \equiv an \frac{D}{d^2} \quad (10)$$

where d is the mean spacing between receptor molecules ($d \simeq 40$ nm for B cells¹⁸), D is the receptor diffusion coefficient¹⁹ ($D \gtrsim 5 \times 10^{-11}$ cm² s⁻¹), nt_D^{-1} is a diffusion limit to the reaction rate and a is the factor by which the actual rate differs from the diffusion limit. For $an \simeq 0.01$, $k_i \rho_0 \gtrsim 0.03$ s⁻¹. We therefore conclude that for antigens which bind to only one site on a receptor molecule, limit

(2) and equation (7) may apply, while for antigens which bind easily to two sites on a receptor molecule, limit (1) and equation (5) probably apply. The precise configuration of epitope binding sites on receptors is not known and it is possible that for some kinds of cells, only one site per receptor molecule may be accessible for binding antigen. It is known that contact with antigen can modulate the number of cell receptors, but such refinements are ignored in the present model.

The model suggests that to achieve response maturation together with multivalent binding, the condition

$$k_d > an \frac{D}{d^2} \quad (11)$$

should be satisfied. Maturation is therefore favoured by (1) large k_d , that is, rapid release of initially bound antigen such as is expected for binding to a single site on the receptor molecule, (2) small D , that is, sluggish diffusion of receptor molecules, (3) large d , that is, large spacing and small density of receptors, and (4) small antigen valence, n . These three first factors may differ between various cell classes, such as T and B cells, or between different stages in cell maturation. There is some evidence²⁰ that cells in an IgM response may not mature whereas those in an IgG response do mature. If so, the model suggests that the cells which are proliferating in IgM(IgG) responses do not (do) meet equation (11). During maturation, a clone might switch from IgM to IgG production and begin to meet equation (11) by changing the number, mobility or accessibility of its receptors.

This model indicates some simple ways in which receptor properties may affect the kinetics of antigen binding to cells and cell behaviour. It also suggests the importance of quantitative measurements of receptor number and mobility and of antigen-to-cell binding kinetics in elucidating the mechanisms which operate in antigen selection of clones, either for amplification or suppression.

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Localisation of sites of GABA catabolism in the rat retina

GAMMA aminobutyric acid (GABA) has been demonstrated in invertebrate and vertebrate retinæ^{1,2,3} and has been shown to have an inhibitory action on retinal neurones⁴. *In vivo* studies of ³H-GABA uptake by rabbit retina have shown a distribution of radioactivity in the inner plexiform layer, the amacrine cells of the inner nuclear layer, the ganglion cell and nerve fibre layers⁵. Further studies have shown that all layers of the rat retina except the outer zone of the rods will accumulate ³H-GABA *in vitro*⁶ in the presence of amino-oxyacetic acid (AOAA), an inhibitor of GABA metabolism⁷. Uptake was most pronounced in the ganglion cell and nerve fibre layer, inner nuclear and outer plexiform layers and at the outer limiting membrane. Furthermore, it was suggested that ³H-GABA might be largely taken up by Müller's fibres and their nuclei rather than by amacrine cells in the inner nuclear layer of the retina.

Other reports have described sites of enzyme activities involved in GABA metabolism in the retina. Glutamate decarboxylase (GAD) was unevenly distributed across the six well-defined layers of the dark-adapted frog retina³ and in layers of the rabbit retina¹. GABA-transaminase (GABA-T) was present in the frog retina in both the light and dark adapted states² although attempts to localise GABA-T at the cellular level in the rabbit retina were unsuccessful¹.

Previous studies have shown the ability of retinal cells to accumulate ³H-GABA, the presence of a GABA-synthesising enzyme (GAD) and correlative amounts of its product in retinal cells¹. If GABA were involved in inhibitory transmission in the retina⁴, there would be a requirement for a GABA-degradatory process. The present histochemical study investigates the localisation of the GABA-degrading enzyme GABA-T in the rat retina.

Intact eyes from adult (250–300 g) Sprague-Dawley rats were removed under ether anaesthesia, embedded in supporting tissue on microtome chucks and quenched in liquid nitrogen; preliminary studies indicated that there was no contamination from the embedding tissue. The chucks were placed in a cryostat compartment and after equilibrium at –18° C, unfixed sections were cut at 12 µm, mounted onto glass slides and freeze dried over activated silica gel at –20° C for a minimum of 3 h. GABA-T activity was demonstrated by a previously described dehydrogenase/tetrazolium-coupled system^{8,9}. In control studies tissue sections incubated with all components except GABA produced a very faint reaction only after extended incubation, which was probably due to NADH₂ diaphorase, an integral part of any tetrazolium-linked system. Sections incubated without α-ketoglutarate also showed a very weak reaction after extended incubation but an intense well-localised formazan precipitate was only seen when all substrate components of the incubation medium (GABA, α-ketoglutarate, NAD⁺) were included. The specificity of this method has been described elsewhere⁸. Routine Ehrlich's haematoxylin and eosin stains were carried out on alternate sections in order to identify the retinal layers.

Light microscopic examination after 30 min incubation revealed that a reaction was mainly present in the ganglion cell and inner plexiform layers, the inner nuclear and photoreceptor layers and the pigment cell layer. The reaction was absent in cells of the outer nuclear layer and in the outer and inner limiting membranes (Fig. 1a). The moderate reaction in the cytoplasm of the ganglion cells of the ganglion cell layer was similar to the reaction seen in the neuroglia in this layer. The strong reaction in the inner plexiform layer corresponded to the site of synapses between bipolar and amacrine cells and the ganglion cells. Some cells of the inner nuclear layer exhibited a good reaction, however, comparison of this stained layer with an H and E stain (Fig. 1b) showed that not all cells in the inner nuclear layer showed the enzyme

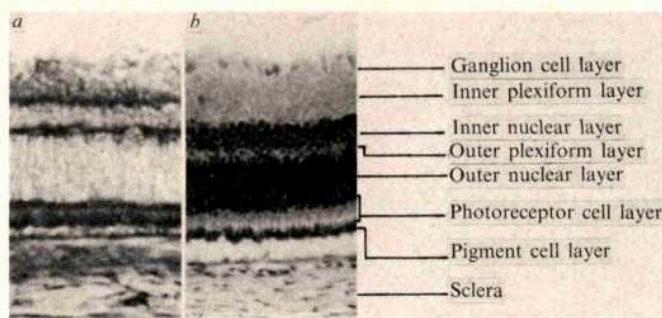


Fig. 1(a), Sites of the GABA-degrading enzymes GABA-T in transverse section of rat retina. Note reaction in the ganglion cell layer, inner plexiform layer, inner nuclear layer and photoreceptor layer (X357). (b), Transverse section of rat retina. Ehrlich's haematoxylin and eosin stain (X357).

activity. A reaction in cells of the outer zone, comprising mostly of horizontal cells, was absent. The reaction was also absent in the bipolar cells of the outer intermediate zone. However, a strong reaction was seen in the zones containing the cell bodies of Müller's fibres (inner intermediate zone) and the amacrine cells (inner zone) (Fig. 2). A reaction was seen in the sustentacular fibres of Müller which extended through the retinal layers between the outer limiting membrane and the ganglion cell layer; the reaction was clearly defined where the fibres terminated in large conical expansions at the vitreal aspect (Fig. 2).

No reaction was seen in the outer plexiform layer at the site of synapses between the rod and cone cells and the bipolar cells. An intense reaction was seen in the inner zone of the photoreceptor layer outside the outer limiting membrane, and in a thin layer of rod and cone cell bodies immediately adjacent to the vitreal aspect of the outer limiting membrane. The reaction was weak in the outer zone of the photoreceptor layer. The epithelial cells of the pigment cell layer and cells of the sclera exhibited a moderate reaction.

Information has accumulated to indicate that the retina possesses a high affinity active uptake system for GABA^{10,11}. It has been suggested that besides uptake and storage of ³H-GABA by 'GABA-neurones'¹⁰ this system also exists for the removal of GABA from retinal neurones or the neuronal environment after release from inhibitory nerve endings¹¹. Other data indicate that the Müller gliocytes of the retina may be largely responsible for inactivation of GABA by this uptake process^{3,6}. We propose that Müller gliocytes are also responsible for the degradation of GABA, which occurs in these cells after GABA uptake and is indicated by high levels of GABA-T activity in Müller cell bodies and fibres running radially throughout the retina. In support of this, intravitreal injection of ³H-GABA failed to produce labelling of Müller cells *in vivo* 4 to 12 h after injection⁵, probably due to catabolism of GABA by the high GABA-T activity now demonstrated in Müller's cells. Inhibition of retinal GABA-T by AOAA however potentiates accumulation of ³H-GABA in the retina⁷, especially in Müller's cells⁶. This strongly suggests that these glial cells are partly responsible for the uptake and degradation of GABA in the retina and that GABA is not stored after *in vivo* uptake into these cells.

Although amacrine cells possess high levels of GABA-T activity they also accumulate ³H-GABA *in vivo*^{5,10} unlike Müller gliocytes. This suggests that a protective store for GABA exists in the amacrine cells; a protective store for GABA is believed to occur in some retinal neurones¹². In this way, GABA is stored for liberation during neuronal activity, possibly during inhibitory action on ganglion cells (for refs see ref. 10). The presence of GABA-T in amacrine cells may act as a limiting factor on GABA storage, although the process for this is obscure and cannot be shown by this study.

An uneven distribution of endogenous GABA, ^3H -GABA uptake, GAD activity^{1,3,5,6,10} and GABA-T activity in the retina has been demonstrated. Within the inner plexiform layer there are high levels of GAD and GABA-T activity. GAD activity is thought to be located predominantly in amacrine nerve endings³ suggesting that the amacrine may be the main GABA synthesising cells of the retina. From present data, GABA-T activity is thought to be localised essentially in the meshwork of Müller's fibres and associated fibrillae which surround the synapses of the inner plexiform layer and not in the synapses themselves. Light microscopy cannot completely resolve this feature but the conclusion is supported by the observation that GABA-T activity in Müller's fibres increases in areas corresponding to synaptic regions, where the fibres pass through the inner plexiform layer.

The presence of high levels of GABA-T activity in the inner zones of the photoreceptor layer and in a thin layer of rod and cone cell bodies at the outer limiting membrane is also intriguing. These zones have very low GAD activity and low levels of endogenous GABA³; they accumulate ^3H -GABA *in vitro* in the presence of AOAA but not *in vivo*^{5,10}. This indicates that GABA-T is probably involved in GABA degradation in these zones. GABA-T activity may be present in the rod and cone cells but the uneven distribution of GABA-T activity and ^3H -GABA uptake indicates that there are specialised areas or structures involved. Fibrillar baskets originating from Müller's fibres at the external limiting membrane form around the proximal parts of the photoreceptor layer and it is possible that these baskets are the main sites of GABA-T activity and ^3H -GABA uptake *in vitro*⁶. The presence of a GABA-T mediated GABA inactivating mechanism in the photoreceptor layer would support

recent evidence that horizontal cells, which synapse with rod and cone cells, have a GABA-related inhibitory function in the retina^{3,13}. The uneven distribution of the GABA system within the retinal layers indicates that the system is compartmentalised between retinal cells. The evidence suggests that GABA synthesis, uptake and storage occurs in neuronal compartments, whilst uptake and degradation of GABA takes place to a large extent in a separate cellular compartment, probably glial. This is in agreement with other investigations into the GABA system in nervous tissue^{3,5,6,10,14,16}.

To obtain further information on the localisation of GABA-T in the inner plexiform and photoreceptor layers, we are currently examining the ultrastructural localisation of GABA-T activity in these areas.

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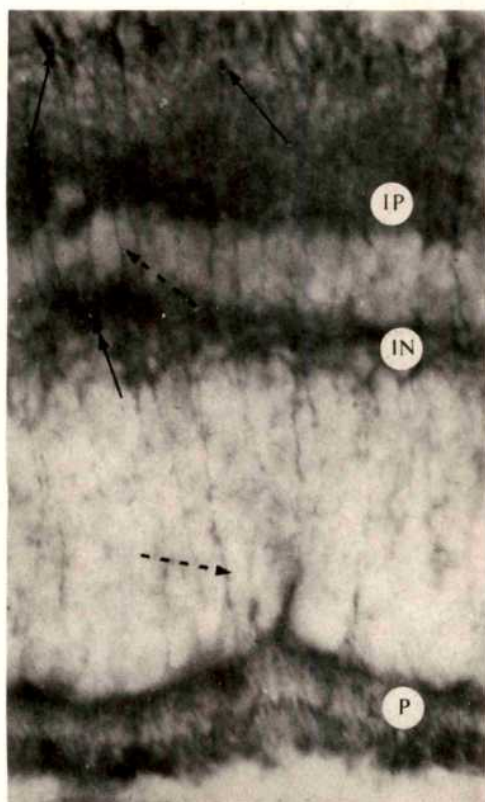


FIG. 2 Sites of the GABA-degrading enzyme GABA-T in transverse section of rat retina. Note strong reaction in Müller's fibres (broken arrows), their cell bodies (double headed arrow) and the conical expansions at the vitreal aspect of the fibres (single headed arrows). A reaction is also present in the inner plexiform layer (IP) and photoreceptor layer (P) and the inner nuclear layer (IN) (x1,750).

Remote nerve fibre bundle alterations in the retina as caused by argon laser photocoagulation

CONSIDERABLE information has been accumulated on the morphological characteristics of retinal lesions produced by laser sources¹⁻⁴. The argon laser is the most widely used energy source for clinical photocoagulation because of its optical and operational qualities.

Laser-induced retinal alterations characterised so far indicate that with increased energy, damaged areas include outer segment layers as well as the pigment epithelium, which is considered the primary site of absorption^{2,3}. Extremely low levels of coherent radiation also produce ultrastructural alterations in sensory retina without apparent change in pigment epithelium⁵. Most reports however have dealt primarily with the lesions or successful treatment⁶⁻⁸. Little information is available on possible secondary sites of injury which may not be spatially or temporally contiguous with the primary lesion. We describe here secondary alterations found considerable distances from the site of primary impact and not occurring immediately.

Single clinical level exposures were placed on retinal vessels as well as vessel-free areas in twelve rhesus monkey (*Macaca mulatta*) eyes. An argon laser, operating at 514.5

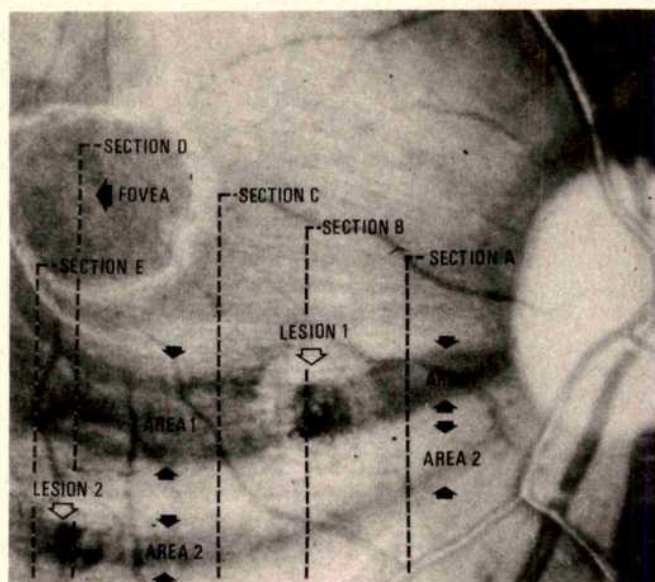


FIG. 1 Fundus photograph of 10-week-old exposures. Lesion 1 was placed at 300 mW, 1 s exposure and 150 μ m spot size. Lesion 2 was placed at 160 mW, 1 s exposure and 150 μ m spot size.

nm and TEM₀₀ mode, was used. The animals were observed for up to 1 year after exposure using direct ophthalmoscopy and fundus photography. Six eyes, from three animals, were enucleated and serial sections, stained with haematoxylin and eosin, were studied under light microscopy. Exposure levels, pulse durations and spot sizes used are given in Table 1.

Immediately after exposure, areas peripheral to the lesions exhibited no ophthalmoscopically visible changes. The colour and capillary network of the optic disk and surrounding tissue appeared normal. Within 5–10 d, however, a darker

area of tissue, of arcuate course and running through the lesions, extended both centrally toward the disk and peripherally away from the lesions, apparently following the distribution of nerve fibres and persisting for 1 yr after exposure (Fig. 1).

Examination of histo-pathological sections, taken 10 weeks after exposure, confirmed the presence of alterations corresponding to the darkened areas. Centrally toward the disk, a diminution of nerve fibres was evident. Other layers of the retina seemed to be unaltered (Fig. 3a). In the area of the lesions, outer segment, inner segment and outer nuclear layers of the retina as well as the pigment epithelium were affected, the extent of damage diminishing with distance from the centre of the impact site (Fig. 3b). Peripheral to the lesion sites, the thinning of the nerve fibre layer was accompanied by a diminution of ganglion cells (Fig. 3c). Again, as was the case central to the lesion all other retinal layers appeared unaltered. This effect is amply demonstrated in Fig. 2, corresponding to the fundus photograph (Fig. 1). Section A is central to both lesions, and compression of the nerve fibre layer, corresponding to the two darkened areas, is evident. Section B is through the site of lesion 1. Section C is peripheral to lesion 1 and central to lesion 2. The effect of lesion 1 shows compression of nerve fibres as well as thinning in the ganglion cell layer, while the effect of lesion 2 remains relatively unchanged from section A. Section D shows lesion 2 and the fovea. Section E is peripheral to both lesion sites and demonstrates the compression of nerve fibres and loss of ganglion cells in both cases. This effect seemed to be more pronounced in lesions placed on vessels than those placed on vessel-free areas.

The implications of this finding are numerous. Since damage to the nerve fibre bundle seems to be involved, the retina is being altered far from the primary site of damage. It has been reported that whenever there are visible nerve fibre defects in the arcuate or radial nerve fibres, corresponding defects are found in the visual field⁹. This type of damage implies both retrograde axonal, as

FIG. 2 Serial sections corresponding to Fig. 1. Section A is central to both lesions. Section B shows lesion 1 and thinning of nerve fibres corresponding to lesion 2. Section C depicts areas peripheral to lesion 1 and central to lesion 2. Section D shows lesion 2 and diminution of nerve fibres and ganglion cells as a result of lesion 1. Section E shows peripheral areas to both lesions 1 and 2.

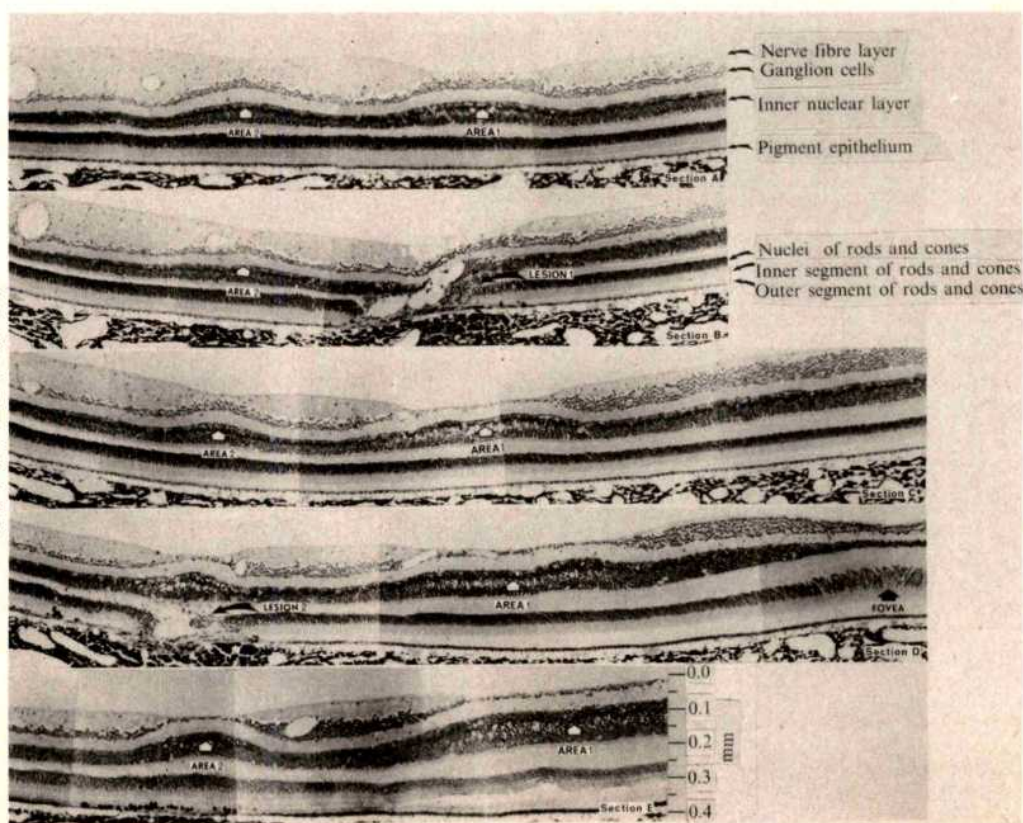


TABLE 1 Power Levels, Spot Sizes, Pulse Durations and Retinal Locations used in obtaining Ophthalmoscopically Visible Nerve Fibre Damage

Power level (mW)	Spot size (μm)	Pulse duration (s)	Retinal location	Ophthalmoscopically visible Nerve fibre damage
125	100	1.00	Retinal vessel	1 of 1
			Vessel-free area	1 of 1
125	500	1.00	Retinal vessel	2 of 4
			Vessel-free area	0 of 1
160	150	1.00	Retinal vessel	1 of 1
300	150	1.00	Retinal vessel	1 of 1
250	50	0.50	Retinal vessel	0 of 1
350	50	0.50	Retinal vessel	1 of 1
250	500	0.25	Retinal vessel	2 of 2
			Vessel-free area	1 of 1
180	150	0.25	Retinal vessel	0 of 1
200	100	0.25	Retinal vessel	2 of 2
			Vessel-free area	0 of 1

well as Wallerian or ascending degeneration. One can generalise that the presence of these ophthalmoscopically visible dark streaks would indicate permanent involvement of optic axons and that the size of the field defect will equal or exceed the area predicted from the size of the retinal nerve fibre bundle damage.

Clinically, nerve fibre field defects have been associated with extremely high retinal energy densities and with repeated photocoagulation about the optic disk¹⁰. Seldom have they been assumed to be associated with initial treatment at moderate power levels. Presumably, the damage is thermal, where the increase in temperature is attributed to the radiation of heat from the underlying pigment epithelium, that is, incident light is absorbed by the pigment epithelium and converted into heat. After the initial photocoagulation, the retina is assumed to become thin so that the nerve fibre layer is in closer proximity to the retinal pigment epithelium causing a greater thermal gradient than in the initial treatment. Clinical power levels range from approximately 300 mW. (2 s pulse, 50–100 μm spot size) used to treat histoplasmosis choroiditis, to 100 mW. (0.1 s pulse, 50 μm spot size) used to treat retinal neovascularisation. Clearly our exposures lie within this range. Since rhesus monkey retina would tend to be more sensitive to laser radiation than that of humans (assuming the previously

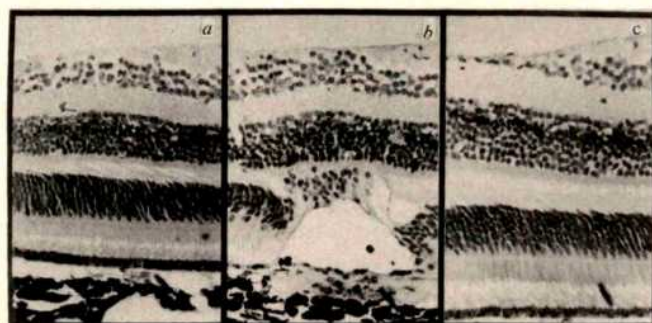


Fig. 3 a, Serial section of area central toward disk. Note the diminution of nerve fibre layer. (200 mW, 100 μm spot size and 0.25 s pulse). b, Peripheral section of impact site. Note the thinning of nerve fibres and involvement of pigment epithelium, outer segment, inner segment, and outer nuclear layers. c, Area peripherally away from lesion. Note absence of nerve fibre layer and diminution of ganglion cells. Other layers of retina seem to be unaffected.

discussed thermal considerations to be true), it is conceivable that this demonstrated effect is not as pronounced in the latter. This finding however does indicate that nerve fibre bundle damage is a distinct possibility at the presently used power levels. Much more work is required to establish the relationship of energy density and pulse duration to nerve fibre damage.

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Temporal pattern of placental lactogen and progesterone secretion in sheep

HUMAN and monkey placental lactogens (PL) have been isolated and characterised biologically and chemically¹⁻³ and reports have appeared identifying a placental lactogen in rats⁴⁻⁶, mice⁷ and goats⁸. More recently rat serum PL concentrations have been determined quantitatively with a radioreceptor assay⁹. In the present study we have used the same assay with minor modifications to identify and measure plasma placental lactogen concentrations in sheep during pregnancy and have compared the secretory pattern of this new hormone with plasma concentrations of progesterone and pituitary prolactin.

We assayed plasma samples obtained at weekly intervals throughout pregnancy in three sheep. Blood samples were collected from animals housed in individual pens by needle puncture from the jugular vein into heparinised tubes. After centrifugation plasma samples were kept frozen at -20°C and some were stored for as long as 2 yr before assay. Lactogenic hormone in these samples was assayed by a radioreceptor assay which has been shown to be specific for lactogenic hormones. The assay uses membranes prepared from mammary glands⁹ of term pregnant rabbits. Plasma concentrations of pituitary prolactin were measured by a homologous, double antibody radioimmunoassay for ovine prolactin (OPRL) using ovine prolactin (NIH-P-S10, 26 IU mg^{-1}) as standard¹⁰. The progesterone concentration in these samples was determined by competitive protein binding assay¹¹.

We also examined in more detail the time course of changes in the concentrations of the three hormones that occur at parturition. Pregnant sheep were bled at intervals of 6 h from 96 h before to 60 h after parturition and the concentrations of all three hormones were determined.

Particulate membrane receptors were incubated with ¹²⁵I-OPRL and displaced by increasing concentrations of

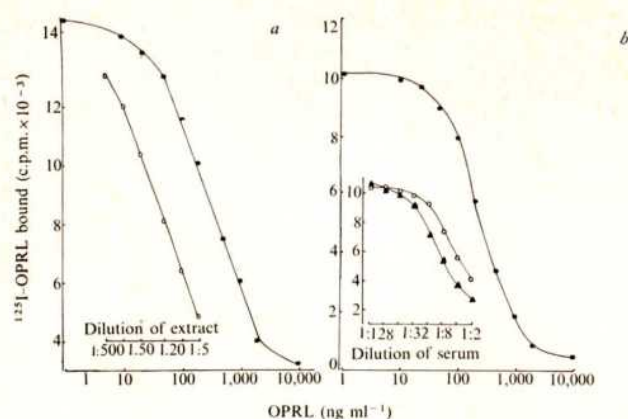


Fig. 1 *a*, Displacement curves obtained when particulate membrane receptors were incubated with ^{125}I -OPRL (100,000 c.p.m.) in the presence of increasing concentrations of OPRL or dilutions of ovine placental extracts. The ordinate represents the amount of ^{125}I -OPRL bound to the receptors expressed as c.p.m. and the abscissa is the concentration of OPRL added to the assay tube. Values for tube blanks were subtracted from the total ^{125}I -OPRL bound to the membranes. The tube blanks were determined by counting the tubes which contained no membranes and were usually 3–4% of the total radioactivity added. The incubation was carried out at room temperature for 6 h. Membrane-bound and free hormone were separated by low speed centrifugation (750g) for 30 min. Foetal cotyledons were extracted for 4 h in 0.1 M NH_4NCO_3 (1 g per 20 ml) centrifuged and dilutions of the extract were assayed. *b*, Displacement curves obtained with serum or plasma samples. The assay procedures were identical to those described in the legend to (*a*), except that 25 μl of either normal human male serum (NHMS), hypophysectomised rat serum or normal sheep serum were added to each of the tubes containing the OPRL standards. When plasma rather than serum samples were assayed, it was necessary to add heparin to the diluting buffer to inhibit clot formation. The inset shows inhibition curves of dilutions of day 114 pregnant sheep plasma and OPRL in the presence of equivalent dilutions of NHMS. The 1:2 dilution was prepared by combining equal quantities of NHMS and OPRL, 2 $\mu\text{g ml}^{-1}$. *a*: ●, OPRL standard; ○, ovine placental extract. *b*: ●, OPRL standard (+ serum); ○, dilutions of day 114 pregnant sheep plasma; ▲, OPRL in presence of dilutions of NHMS.

unlabelled OPRL (Fig. 1). The curves obtained with dilutions of ovine placental extract were parallel to the OPRL inhibition curves (Fig. 1*a*). Despite concentrations of lactogen greater than 8 $\mu\text{g ml}^{-1}$ in the placental extract, the radioimmunoassay for pituitary prolactin failed to detect any lactogen in these extracts. The displacement curve for serum or plasma assays is shown in Fig. 1*b*. In this assay procedure the prolactin standard contained 25 μl of normal human male serum (NHMS). The inset shows the parallelism of dilutions of day 114 pregnant sheep plasma and OPRL in the presence of equivalent dilutions of NHMS.

Figure 2 shows the plasma concentrations of PL, prolactin and progesterone in samples from three ewes. Placental lactogen can usually be detected in plasma samples by day 60 of gestation and thereafter it increases as pregnancy advances, reaching peak concentrations of 1,000 to 2,000 ng ml^{-1} on days 95 to 114 of gestation. After the initial peak there is generally a decline in PL concentration followed by one or more peaks before parturition. These fluctuations, especially in ewe 187 (Fig. 2*c*) are reproducible and not due to assay variation, as similar overall patterns were observed on re-assay of the same plasma samples. The plasma concentrations of pituitary prolactin measured by radioimmunoassay remained below 50 ng ml^{-1} until a few days before parturition. So, except at term, the concentrations of PL measured by radioreceptor assay in plasma samples of pregnant sheep cannot be ascribed to the concentrations of prolactin of pituitary origin which is in the circulation.

Progesterone concentrations in two of the three animals very closely paralleled the temporal pattern of PL concentrations. In ewe 187 during the second half of pregnancy several peaks of PL were noted, only one of which exceeded 1,000 ng ml^{-1} . In spite of this, the progesterone concentrations in this animal were similar to those of the other two ewes.

The plasma concentrations of PL, prolactin, and progesterone at the time of parturition in three sheep are shown in Fig. 3. Placental lactogen concentrations slowly declined from approximately 1,000 ng ml^{-1} to 500–700 ng ml^{-1} by 12 h before parturition; they then decreased quite rapidly *post partum*. Plasma concentrations of pituitary prolactin began to rise about 36 h before parturition, reaching levels of 300–600 ng ml^{-1} approximately 3–6 h before parturition. At this time the PL concentrations measured by radioreceptor assay were almost entirely due to the cross reaction of pituitary prolactin in the receptor assay. During this time progesterone concentrations also fell rapidly together with PL concentration. In two of the three sheep the decrease in progesterone preceded that in PL by 6–12 h.

To estimate the relative halftime rate of disappearance of PL, the uterine vessels of two ewes were ligated on day 140 of pregnancy and plasma samples were collected. Assay of these samples by radioreceptor assay showed that PL disappeared from the circulation with a halftime of less than 20 min.

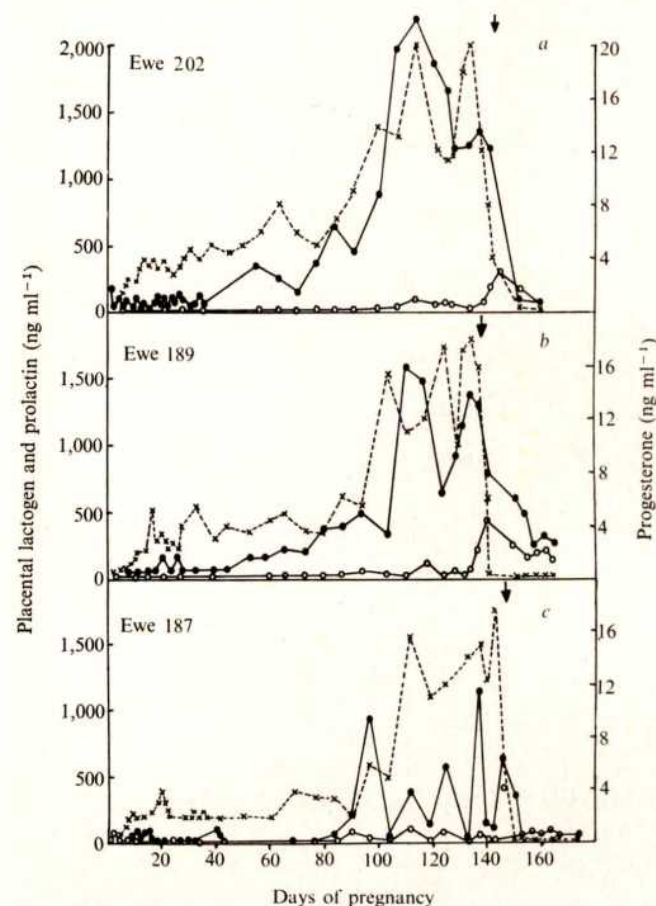


Fig. 2 Plasma concentrations of placental lactogen (PL) were measured by radioreceptor assay, plasma concentrations of pituitary prolactin (PRL) were measured by radioimmunoassay, and progesterone levels were determined by competitive protein binding assay during pregnancy in three sheep. The radioreceptor assay detects both PL and pituitary prolactin, however, except at term, the plasma concentration of the latter is trivial by comparison with PL levels. The radioimmunoassay for pituitary prolactin fails to detect placental lactogen. The arrow (↓) indicates the day of parturition. ●, Placental lactogen; ×, progesterone; ○, prolactin.

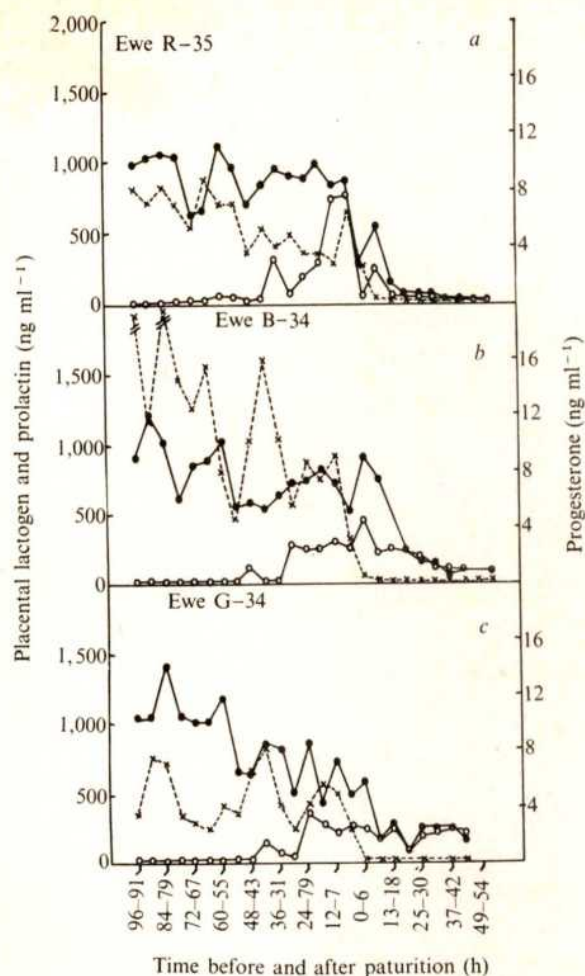


FIG. 3 Placental lactogen, prolactin and progesterone levels from 96 h before to 60 h after parturition in three sheep. Parturition occurred at some time between the intervals 6-0 and 0-6 h. ●, Placental lactogen; ×, progesterone; ○, prolactin.

Both the foetal and maternal cotyledons were extracted in 0.1 M NH_4HCO_3 . The concentration of PL in both extracts was 200 μg per wet weight of tissue which is similar to the concentration of PL human placental extracts². After fractionation of ovine placental extracts on Sephadex G-100, PL appeared in the same elution volume as ^{125}I -OPRL which had been added as a marker, suggesting that the molecular weights of PL and pituitary prolactin are very similar.

Several hormonal changes have been documented at the time of parturition in sheep. These include sequentially, an increase in blood cortisol in the foetus¹², followed by an increase in total unconjugated oestrogens¹³ and in the individual unconjugated oestrogens¹⁴ and a decrease in serum progesterone in maternal blood¹⁵ and, as we show here, a parallel decline in PL concentrations. The precise interrelationship and significance of all these hormonal changes is still obscure. Nevertheless, the general similarity of the temporal pattern of plasma concentrations of PL and progesterone is sufficiently intriguing to suggest that PL may stimulate progesterone production. With the purification of ovine PL in adequate amounts it should be possible to test this hypothesis directly. The fact remains that a new PL has been identified and quantified in plasma and placental extracts of pregnant sheep and this observation offers a new model to study the elusive role of placental lactogen.

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Cortical hypothermia is a sequela of electroconvulsive shock

THE impaired retention of a recently learned habit that is commonly observed in experimental animals after administration of electroconvulsive shock (ECS) is also observed after partial immersion for a few minutes in cold water¹⁻⁴. The physical differences between a hypothermal and an electrical amnesic agent do not preclude a common mode of action; indeed, we have confirmed that ECS results in hypothermia as well as amnesia.

Eight mature, partially pigmented rats were implanted surgically, under barbiturate anaesthesia, with two 2.5 mm stainless steel screw electrodes that were used to administer ECS and also to record the electrocorticogram (ECOG). In each rat electrodes were threaded through small bur holes in the calvarium to contact the dural membrane about 5 mm lateral to the midline, one electrode to the frontal cortex and one to the contralateral occipital cortex. A third electrode was screwed into the calvarium at the midline about 10 mm anterior to the bregma suture; this later served as an electrically indifferent pole for recording the ECOG. Each rat was also fitted with a small thermistor (United Systems model 430) that was lowered through a bur hole in the calvarium and then through an incision in the meninges to rest on parietal cortex near the vertex. Insulated conductive leads from each of the screw electrodes, and from the two leads of the thermistor, were dressed closely to the calvarium and then individually terminated at one of the five connector pins of a small socket anchored to the calvarium by dental cement. For a minimum of 7 d after surgery, the rats were housed continuously in individual cages in a vivarium where the ambient temperature was maintained between 22° and 26°C,

and relative humidity was between 50 and 70%. Purina Lab Chow was available *ad lib* in the home cage; so too was drinking water, until commencement of pretraining.

TABLE 1 Numbers and means of entries of ten control and eight experimental rats during formal training trial of the tenth day and during the retention trial of the twelfth day.

	Control			Experimental		
	Pre-ECS*	Post-ECS†	Diff.‡	Pre-ECS*	Post-ECS†	Diff.‡
17	28	11		12	11	-1
9	21	12		12	9	-3
7	17	10		10	19	9
6	21	15		9	13	4
5	21	16		6	14	8
5	20	15		5	7	2
5	13	8		3	4	1
4	32	28		3	4	1
3	24	21				
2	18	16				
M =	6.3	21.5	15.2	7.5	10.1	2.6
	$P < 0.001$			$P > 0.05$		
				$P < 0.001$		

* Formal training trial of tenth day.

† Retention trial of twelfth day.

‡ Difference between number of Post-ECS and Pre-ECS entries.

Our measure of amnesia was a one-trial appetitive learning task developed by Tenen⁵ and independently confirmed^{6,7} as a mnemonic assay that controls for possible aversive sequelae of ECS. The task initially involved 10 successive days of pretraining during each of which, after 22 to 24 h without water, a rat was permitted to explore for 5 min the interior of an open-topped enclosure containing a nook in one wall. During all trials of pretraining, formal training and testing for retention, a set of flexible conductive leads was fastened to a rat's calvarium-pedestal; the shape of the nook accommodated the leads in such a way that an animal could enter and leave it without restriction. The formal training trial was conducted during the eleventh day, the only day in which a drinking tube that provided water was present in the nook. Fifteen seconds after each animal had begun to drink it was removed from the enclosure and was held while 40 mA (root-mean-square) of 60 Hz alternating sine-wave current was passed between the frontal and occipital screw electrodes for 0.5 s. Ten control rats, which shared a common history of origin, housing, surgery and pretraining, received sham-ECS treatment—no electrical current—but were otherwise handled in the same way. During the twelfth day, 22 to 24 h after ECS or sham ECS, each of the eighteen rats was returned to the enclosure and observed for 5 min, as under pretraining conditions, and its entries into the nook were counted.

The averaged number of entries of the ten unshocked rats of the sham-ECS group increased more than three-fold between the tenth day (6.3) and the twelfth day (21.5); on the twelfth day they behaved as if they expected to find water. In contrast, the eight shocked animals performed during the twelfth day about the same as they had during the tenth, respectively, averaging 10.2 and 7.5 entries; they seemed to remember little about the previous availability of water in the nook (Table 1). These findings were evaluated by subtracting the number of entries made by each rat during day 10 from the number it made during day 12; the *t* test for independent samples was then performed on the means of the difference scores, which were 2.62 and 15.20, respectively, for shocked and unshocked rats. The shocked group gave highly reliable evidence of poorer retention (*t* ratio = 5.13 at 16 degrees of freedom; $P < 0.001$).

The observations of primary importance to our study began just before the formal training trial of the eleventh day with a single 5 min recording of the baseline temperature

and then of the ECoG. Independent sets of flexible leads to a precision digital electronic thermometer (United Systems model 1502) and to a polygraph (Grass model 6-8-2) were attached sequentially to the calvarium-pedestal of each rat and then, after the thermal and the ECoG baselines had been recorded, were disconnected for attachment of the ECS leads. After the training trial and administration of ECS, alternate recordings of temperature and of the ECoG were made from time to time until the temperature of each rat was observed to increase to its baseline value. Cortical temperatures, which ranged from 36.4 to 38.0°C and averaged 37.2°C before ECS, decreased rapidly after the ECS treatment. The nadir of most of the shocked rats' temperatures occurred in about 15 min, ranged from 0.95° to 2.46°C below individual baselines, and persisted for about 30 min before a trend toward recovery was observed (Fig. 1). In all but one animal recovery to the baseline temperature occurred within 120 min. The one rat, however, exhibited a fluctuating hypothermia that persisted for nearly 10 h. Several days later, two of the eight experimental rats were selected randomly to provide additional baseline thermal data and were again subjected to the procedures of the eleventh day, except that they received sham ECS treatment. The brain temperatures of both animals during the 60 min recording session that followed were close to original baselines (Fig. 1).

When pre-ECS and post-ECS records of the ECoG and of the brain temperatures of individual animals were examined and compared, there was a marked covariation across time between thermal and electrocortical activity. The medium amplitude electrical activity of mixed, low-and-high frequencies that characterised the ECoG before ECS changed to the spiking and high-amplitude slow waves that characterise the electroencephalogram immediately after ECS⁸⁻¹⁰. A bout of isoelectric activity often followed the spiking and slow waves. Subsequently, until the cortical temperature began to increase, the ECoG was predominately one of slow wave, deltoid patterns. With initiation of thermal recovery there was a noticeable trend toward normalisation of the ECoG. Segments of the ECoG of a representative rat (Fig. 2) confirm to the eye the close correlation between electrical activity and temperature of the superficial cortex.

The major findings of our study are that: (i) a reversible cortical hypothermia is a consequence of an amnesia-inducing ECS; and (ii) the hypothermia is associated with an alteration of electrocortical activity. The degree of hypothermia

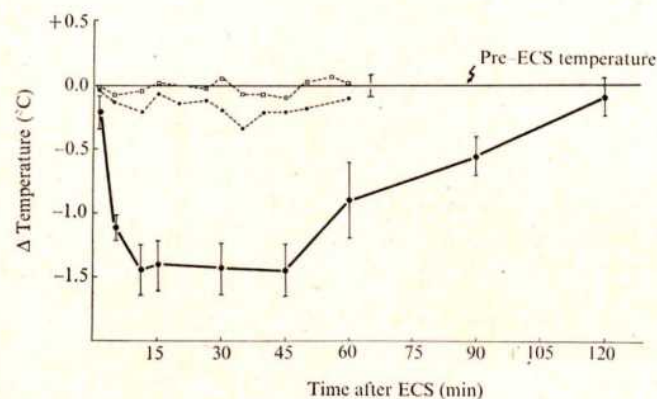


Fig. 1 The heavy line represents averaged decrements in degrees Celsius of cortical temperatures of eight rats after administration of a single electroconvulsive shock (ECS). The baseline of 0.0 °C is based on the averaged stabilised value of pre-ECS (basal) temperatures of the same animals. The dashed lines are individual thermal control data from two of the same rats as measured several days later after a sham-ECS treatment. The vertical lines extend one standard error above and below each mean.

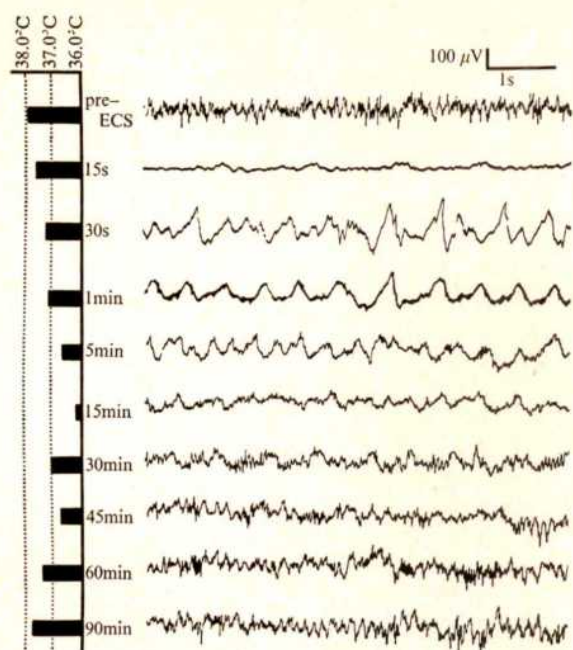


FIG. 2 Representative segments of the electrocorticogram (ECoG) of a rat are shown in relation to its cortical temperature as recorded within a few seconds of each other and as sampled across time before and after a single electroconvulsive shock. The ECoG was recorded from the superficial frontal cortex and was referred to an indifferent electrode near the midline of the cerebrum.

that was exhibited by our rats after ECS seems to be less severe than that which has been reported as necessary to produce amnesia by partial immersion of the murine animal in cold water¹⁻⁴. For example, Beitel and Porter reported² that a reduction of core temperature to 2° C was necessary to achieve reliable amnesic effects in mice. In contrast, Vardaris *et al.* found⁴ that a reduction of the rat's core temperature to 21.5° C produced not only retrograde amnesia but also frank seizure-like activity in the ECoG. In neither study, however, was cerebral temperature measured. Indeed, the absence of published information on simultaneously recorded thermal data of brain and core from animals that have been subjected to, and confirmed by behaviour, for amnesic treatments renders moot the question of a possible lack of parity in the degree of cortical hypothermia. That there is a highly reliable if moderate cortical hypothermia in the rat after ECS is both warrantably assertable and provocative of a more general question: do other amnesic agents such as the cortical¹¹ and hippocampal¹² spreading depressions also result in cerebral hypothermia? An affirmative answer would suggest that cerebral hypothermia is a causal antecedent and not merely a correlate in the stream of events whereby memory of recent occurrences is impaired by ECS and other amnesic agents. If cerebral hypothermia *per se* is critical to the annihilation of a newly forming engram, artificial maintenance of normal temperatures of experimental subjects immediately after ECS—achieved, for example, by immersing them partially in warm water or by carefully dosing them with microwave energy¹³—should preserve the engram. A corollary of the argument, that a mild hyperthermia may facilitate formation and maintenance of the engram, has already gained some support in the literature^{13,14}.

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Short term memory and the EEG

STUDIES of human short term memory and physiological state have produced what seem to be paradoxical results, as efficient performance has been associated with low states of activation. This effect was first demonstrated by Kleinsmith and Kaplan¹ who measured electrodermal response (EDA) to paired associates. As yet, no similar relationship has been demonstrated for EEG activation and memory. Previous studies have been concerned merely with the gross differentiation of task activity and rest and simply show greater activation during learning^{2,3}. No-one has reported a detailed study of changes during verbal learning or of their relationship to subsequent recall.

The EEG was monitored during the acquisition and immediate recall of 9-digit strings presented in the auditory mode. After an initial 2 min rest period, 32 subjects each underwent 24 trials, for which instruction was minimal. The EEG was recorded, stored and averaged, using low frequency analysis in the manner described in earlier studies⁴ to yield an average abundance value per serial position. Because of the limitations of low frequency analysis, inter-digit interval within a series was 3 s. Each trial lasted 45 s with an EEG sample for each of the 15 × 3 s intervals (9 × 3 s per series, plus 3 s warning, plus 15 s for recall and inter-trial rest). The results reported here are largely concerned with 11.5–12.5 Hz; a complete report will be presented later for theta, beta and the remaining alpha frequencies.

Our key results were as follows. Task errors in recall increased monotonically as a function of serial position (ANOVA; $P < 0.001$) with an improvement in performance

for the final serial position ($P < 0.05$). Such an error curve is typical for tasks of this nature. At the same time, EEG abundance during acquisition diminished with serial position, such that high error in subsequent recall was associated with decreasing abundance (increasing arousal) in acquisition (ANOVA; $P < 0.01$). The monotonicity of the decrease in abundance (as assessed by Kendall's S) was positively correlated with number of recall errors in each of the serial positions, the strongest effects holding for the earlier positions (Spearman's correlations by ranks (ρ); $P < 0.05/0.01$). Similarly, total abundance during acquisition was positively related to subsequent recall (Pearson product-moment correlations; $P < 0.01$).

When subjects were divided into high and low recall error groups, the high recall error subjects showed greatest decreasing abundance in both the later stages of acquisition and at recall (ANOVA; $P < 0.05$). Both high and low error groups yielded recall curves which were identical in shape and parallel. But subjects with high recall errors even showed lower abundance (7.5–13.5 Hz) during a pre-test resting period and mean dominant frequency⁵ at that time correlated positively with subsequent error rate (Spearman ρ ; $P < 0.05$).

A within-subjects analysis showed that both high recall error trials and low recall error trials began with a monotonic decrease in EEG abundance (increasing arousal); but good performance (low error) trials then departed from this trend and were associated with higher abundance (t test; $P < 0.02$). The first eight trials were associated both with high recall errors in the early serial positions (ANOVA; $P < 0.01$) and decreased abundance when compared with the final 16 trials (ANOVA; $P < 0.05$). Finally, for all subjects, EEG abundance during between-trial rest was greater than during acquisition and recall ($P < 0.01$).

So far as we are aware, this is the first study to plot the systematic changes which occur in the human CNS during acquisition of material to-be-recalled, and to relate such changes to subsequent recall.

These results were wholly consistent for every type of analysis (subjects before the task, subjects during the task, serial position, high and low recall error trials, trials over time) since poor performance was associated with increased EEG arousal, even to the extent of enabling prediction of recall error rate before the task proper. The results were also compatible with those of Kleinsmith and Kaplan¹ who showed that in paired-associate learning, items which were associated with high EDA arousal were less well recalled in the short term. The finding that the EEG is more activated during the trials compared with the inter-trial resting periods, corroborates earlier work^{2,3}. Within the range of this increased activation, however, superior performance is associated with relatively lower activation levels.

Thus, short term memory tasks are an exception when compared with other tasks demanding sustained attention, such as vigilance tasks, where efficiency is positively related to level of activation. Short term memory tasks of the digit span type, typically demand brief periods of undivided attention, after which the subject may relax and prepare himself for the next trial. Such conditions of testing may well require lower levels of initial arousal to ensure efficiency. It should be noted that in the present task, ample time was available for rehearsal and for the self-monitoring of success or failure in retention.

A provisional interpretation of our results is that subjects who were already more highly aroused were less suited to good performance than subjects with lower initial arousal level. In addition, knowledge of failure in retention may have augmented the trend to increased arousal which in its turn may have reduced further the quality of performance.

The immediate memory span test is long-established within the repertoire of psychological measurement, with many

decades of use in intelligence testing. Further experimentation using the present paradigm may lead to an increase of our understanding of the neurological substrates of intellectual functioning.

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Visual attention affects brain blood flow

THE brain is extremely dependent on an uninterrupted supply of blood-borne nutrients because of its high metabolic rate. The effect of cerebral activity on the provision of blood to the brain, however, is not fully understood, particularly with respect to higher mental function. For human subjects, there is conflicting data concerning the possibility that intellectual effort increases the rate of flow of blood through the brain^{1,2}. We now report that cerebral blood flow may in part be regulated by the extent of mental activity. Our experimental animal was the chick, which has an anatomy such that minor changes in cerebral blood flow are readily detectable.

As the fibres of the optic tract are totally crossed over at the chiasm, each eye of the bird innervates only the contralateral optic lobe³. The cerebral hemispheres constitute the major associative areas of the chick and the innervation of each eye to these hemispheres, although indirect, is predominantly contralateral⁴. Furthermore, the absence of a major interhemispheric commissure, the corpus callosum, reduces interactions between the two symmetrical halves of the brain⁵. Consequently, the metabolic and vascular deficits caused by unilateral visual deprivation are largely confined to regions contralateral to the treated eye^{6,7}. Rapid and widespread changes in the rate of brain blood flow can be induced in chicks by occlusion of an eye by sewing the eyelids shut^{8,9}. However such studies cannot show whether reduced blood flow is due to reduced intensity of sensory input or to a decrease in behaviourally relevant information.

In our experiments two small cardboard blinkers were glued to the heads of 3-d-old chicks that had been fasted for the preceding day. The blinkers were positioned adjacent to each eye so that one eye could see only forward and the other only backward. Chicks were then placed in a smooth,

TABLE 1 Relative blood flow rates in the two sides of chick brain

Ln F/B			
(a) 10 min pecking	Optic lobes	+ 0.093 ± 0.021	($P < 0.001$, $n = 31$)
	Cerebral hemispheres	+ 0.068 ± 0.021	($P < 0.005$, $n = 31$)
(b) 10 min non-pecking	Optic lobes	+ 0.021 ± 0.023	(NS, $n = 23$)
	Cerebral hemispheres	+ 0.018 ± 0.026	(NS, $n = 23$)

F, B, Blood flow through regions contralateral to forward and backward-looking eyes respectively (c.p.m. mg^{-1} tissue). Results are the mean of n birds ± s.e.; P , probability (Student's one-tailed t test); NS, non-significant.

TABLE 2 Difference in blood flow ratios of equivalent regions of pecking and non-pecking chicks

	(ln F/B) pecking—(ln F/B) non-pecking	
Optic lobes	+ 0.072 ± 0.031	(<i>P</i> < 0.025)
Cerebral hemispheres	+ 0.050 ± 0.034	(<i>P</i> = 0.08)

F, B, P are explained in Table 1. Figures are means of differences ± s.e.

white, circular bowl (25 cm diameter, 13 cm high) containing grains of chick feed. Under these conditions all birds immediately began to feed with straight, forward pecks, using the forward-looking eye to locate the grain. After 10 min of feeding, the relative rate of blood flow through the left and right optic lobes and cerebral hemispheres was determined by intracardiac injection of ^{125}I -iodoantipyrine, which is freely diffusible through both aqueous and lipid media¹⁰. After 10 s, the chick was decapitated and the brain rapidly dissected, weighed and placed into scintillation vials for assay of total radioactivity⁸. Data are expressed as c.p.m. mg⁻¹ tissue (wet weight).

Results were calculated as the ratio of the specific radioactivity in the brain region contralateral to the forward-looking eye (F) relative to the corresponding value for the paired region contralateral to the backward-looking eye (B). To avoid skewing data, the natural logarithm of this ratio was determined for each chick (ln F/B). This value was significantly above zero for paired optic lobes and also cerebral hemispheres (Table 1). Thus, the regions innervated by the forward-looking eye had a significantly greater rate of blood flow than regions associated with the backward-looking eye. This occurred even though the intensity of light exposure was very similar for each eye.

The experiment was repeated on a second group of chicks under identical conditions, except that the bowl was devoid of grain. In this situation, chicks do not peck or attempt to peck. The animals were occasionally prodded gently to encourage them to keep their eyes open. Two birds that did not keep their eyes open were not used. When birds had been in the bowl for 10 min, regional cerebral flow was again determined as in the grain-pecking group (Table 1). In this case there was no significant difference in blood flow through regions innervated by the forward-looking eye relative to the backward-looking eye.

A statistical comparison was made between data derived from birds pecking at grain relative to birds not receiving grain (Table 2). The asymmetry of regional blood flow in the pecking chicks was also significantly greater than corresponding values for non-pecking chicks. Thus the production of a difference in blood flow in the two sides of the brain was demonstrable only when the chicks were given grain to peck. Because pecking grain is not a learned behaviour, some other behavioural factor, such as attention or interest must be involved.

Such visual attention is sufficient to increase the rate of blood flow by 7–9% in the directly innervated optic lobes, and in the secondarily innervated cerebral hemispheres. These regions account for more than 75% of the total brain weight in the chick.

Many reports suggest that increased rates of macromolecular synthesis by brain are associated with learning or with exposure to an enriched environment^{11–13}. As attention is a significant feature of all learning and enriched-environment studies, the phenomena described here may underlie some of the metabolic events related to these processes. Such an association could be explained by variations in the supply of metabolites to the brain as the result of changes in brain blood flow. Such vascular mechanisms could also mediate the known relation between the quality of sensory input and cerebral development.

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Bioconvection patterns in swimming microorganism cultures as an example of Rayleigh-Taylor instability

RATHER definite spatial patterns are observed in cultures of swimming microorganisms which are heavier than water and which tend to propel themselves toward the upper surface in response to some external stimulus; for example, gravity, light and chemicals. These patterns are characterised by falling fingers containing the organisms and are maintained by return upward swimming of the organisms. The patterns have been termed 'bioconvection' patterns¹ and have been known since 1848²; they are found in bacterial, flagellate^{3,4}, plankton⁵ and ciliate⁶ cultures.

Wager² studied the patterns formed in cultures of the flagellate *Euglena viridis*, among others, and was able to show that the tendency for these organisms to swim against gravity (negative geotaxis) was necessary for the pattern formation with live organisms. When such organisms are dead and form a layer at the bottom of the culture, a similar pattern of falling fingers can be obtained provided a sealed airless chamber of such a culture is very carefully inverted². In this case, of course, the pattern is not maintained.

Bénard (thermal) instability has been considered as a mechanism for this pattern and has been disproved¹. Indeed, the patterns are observed even when a stabilising temperature gradient is imposed. Another mechanism which has been considered and invalidated⁷ is the turnover effect, which is common in sucrose gradient preparations for centrifugation.

Quantitative observations have been made recently⁷ of the development of bioconvection patterns in cultures of *Tetrahymena pyriformis*, a ciliated microorganism which ex-

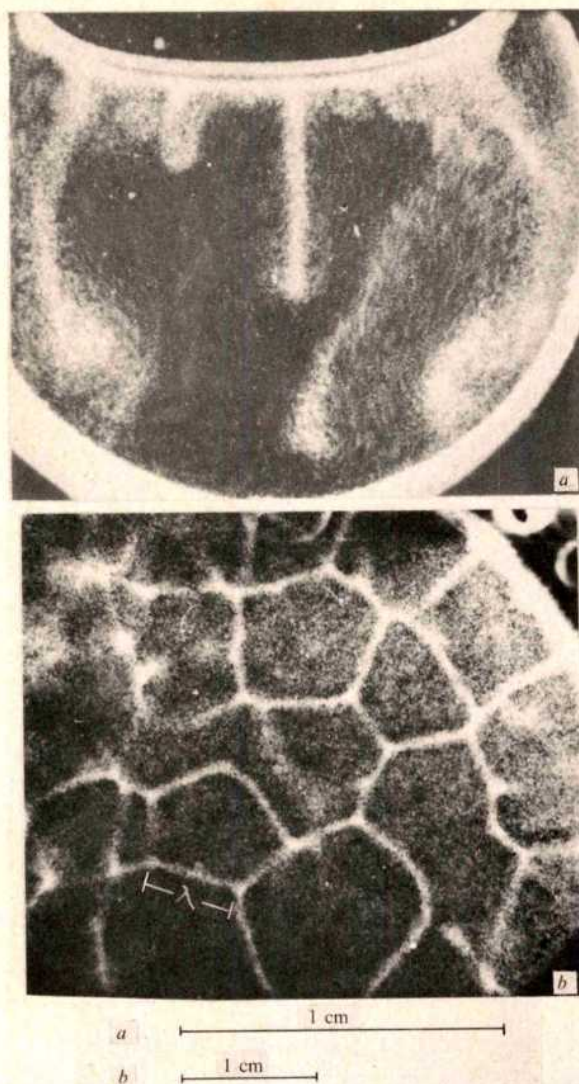


FIG. 1 *a*, A strobe-illuminated photograph of bioconvective sedimentation fingers descending from precipitation nodes; view is from the side. This *Tetrahymena* culture is enclosed in a 0.15 cm thick perfusion chamber of diameter 2.1 cm. The multiple flash mode utilised here gives one an impression of the bioconvective motion. *b*, Precipitation nodes and cross connections in a *Tetrahymena* culture as viewed from above. All light areas are masses of cells reflecting light. White lines are connections, and junctions are precipitation nodes.

hibits negative geotaxis. The system of falling fingers which characterises the pattern follows a period of accumulation in the upper layer of the culture. This layer typically develops nodes or bulges downward with fairly regular separations. The bulges then grow into falling fingers which descend with a velocity approximately twice the normal swimming velocity of the ciliate. Eventually flow connections develop between the nodes in the upper layer, particularly at the higher organism concentrations. The resulting familiar bioconvection pattern is shown in Fig. 1*a* which is a side view and Fig. 1*b* which is a view from above.

When the patterns are fully established the upper layer typically has a thickness of the order of 1.5 mm. It can be deduced readily from ref. 7 that the concentration of organisms in this upper layer is so large that the average distance between organisms is small compared with the layer thickness. As we shall see, the distances between the organisms is also small compared with other possible lengths involved in the physical model with which we are concerned here.

In this physical model the upper layer is a homogeneous

fluid with an excess density determined by the presence of organisms which are heavier than the medium. Recent measurements⁷ lead to values of the density increments for the upper layer over the lower layer and, typically, density increments are found to range from $\Delta\rho = 1.21 \times 10^{-4}$ to $\Delta\rho = 1.1 \times 10^{-3} \text{ g cm}^{-3}$.

The physical view of the process which we adopt here is as follows. As the microorganisms swarm beneath the upper surface, they form a fairly well defined layer of thickness h , which has a density ρ' which exceeds the density ρ of the underlying culture by a small amount $\Delta\rho$. This upper layer is taken to be a homogeneous fluid even though it contains microorganisms. As has been indicated, the approximation of homogeneity is justified for the situation in which the organisms are numerous and uniformly distributed so that their separations are very small compared with the thickness of the layer and with other lengths involved in the physical model such as λ (the internodal distance defined below).

The physical situation is gravitationally unstable, of the sort which is familiar in fluid mechanics as Rayleigh-Taylor instability⁸. The unusual feature of the present conditions is that the gravity force is so small that viscosity becomes quite important. As is usual in a stability problem, the initial growth of a small disturbance is analysed, and for the present unstable case an initial disturbance grows like e^{nt} , where t is the time and n is a function of the wavelength of the disturbance. The exact treatment of this two-fluid problem, where the upper fluid has a small thickness, h and the lower fluid a large thickness has apparently not yet been published, but it is a relatively straightforward matter which, however, has considerable algebraic com-

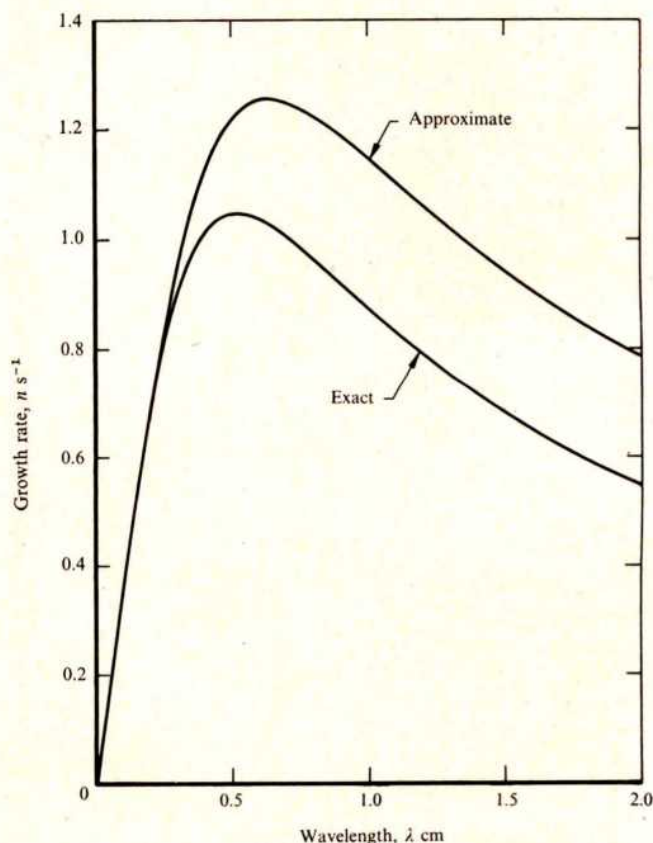


FIG. 2 The ordinate gives n , the growth parameter of the initial interface wave disturbance which has amplitude increasing as e^{nt} . The maximum n is at $\lambda_m = \lambda_{\text{theor}}$ which occurs here at 0.65 and 0.525 cm. These theoretical curves are obtained for $\Delta\rho = 9.0 \times 10^{-4} \text{ gm cm}^{-3}$ with an upper layer depth of $h = 0.13 \text{ cm}$. The λ_m condition corresponding to this model is illustrated in Fig. 3.

TABLE 1 Measured and theoretical values for two *Tetrahymena* cultures

C_0 (cells cm^{-3})	3×10^4	2.71×10^5
h (cm)	0.15	0.13
C_l (cells cm^{-3})	0	5.6×10^5
C_u (cells cm^{-3})	7.1×10^4	1.4×10^6
$\Delta\rho$ (g cm^{-3})	1.21×10^{-4}	1.08×10^{-3}
λ_{meas} (cm)	~ 1.0	0.655
λ_{theor} (cm)(exact theory)	0.80	0.525
λ_{theor} (cm)(approximate theory)	1.1	0.65

The range of parameters shown is characteristic of *T. pyriformis* culture systems and although stated values of C_0 are exceeded in both directions no nodes appear at values below the smaller figure and cultures at values above the larger figure were not analysed. The symbols are defined in the text.

plications. Although the exact solution of this problem has been achieved⁹, the results are available in numerical form only. The results of the exact calculation for a layer thickness $h = 0.13$ cm and a density excess in the upper layer $\Delta\rho = 9.0 \times 10^{-4}$ g cm^{-3} is shown in Fig. 2. It can be shown⁹ that a simple approximation for the amplitude behaviour of a disturbance $a_k(t) = a_k(0)e^{nt}$ is given by

$$n^2 + 2\nu k^2 n - \sigma^2 = 0 \quad (1)$$

where

$$\sigma^2 = \frac{\rho' - \rho}{\rho' + \rho \coth kh} gk \quad (2)$$

Here $k = 2\pi/\lambda$ is the wave number, g is the acceleration of gravity and λ is the internodal wavelength. Figure 2 also shows n as a function of λ as determined from the approximate theory. Both the exact and the approximate theory show that n has a maximum at about the same value of λ . It is evident that there is a relatively small band of wavelengths about this maximum which leads in the growth of the fingers. The λ for which n is maximum, that is λ_m , should give the most frequently observed separation distance between the fingers. While the theory is limited to small amplitudes, it is clear that the wavelengths which lead initially will continue to lead.

For comparison with these theoretical ideas *T. pyriformis* was grown axenically and cultures were poured into dishes 4×5 cm in cross section. Fluid depth was maintained at 1.2 cm. Culture samples from the upper and lower layer were extracted by a device similar to that described previously⁷. Sample concentrations were determined with a Sedgwick-Rafter counting chamber and culture concentrations with a Coulter counter. Swarm layer thickness, h , was determined from photographs. The density increment was found from

$$\Delta\rho = (C_u - C_l)(m_T - V_T\rho_M),$$

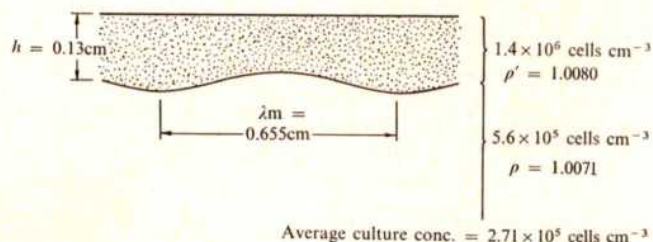


FIG. 3 Rayleigh-Taylor instability parameters measured for one culture of *T. pyriformis*. The $\lambda_m = 0.655$ is an average nearest internodal distance obtained from nodes at least 1 cm from the chamber wall.

where C_u and C_l are upper layer and lower layer medium cell concentrations respectively, m_T and V_T are the mass and volume respectively of *T. pyriformis*, and ρ_M is the culture fluid density. Parameters for a sample culture are presented in Fig. 3.

Table 1 summarises two sets of measurements near the extremes of the observed culture concentration range. The table also gives a comparison of the observed internodal distance with the values predicted by the present theory.

The agreement between the theoretical and observed maximum values is good. We conclude that Rayleigh-Taylor instability is the mechanism for the observed bioconvection in *T. pyriformis* cultures and may also explain sedimentation patterns in other microorganism swarms insofar as they are not disturbed by surface wind or by thermal gradients.

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Decrease of polysomes in *Tetrahymena* after synchronisation shocks

THE rate of protein synthesis in eukaryotic cells can change rapidly as a result of translational control such as the inhibition of protein synthesis during mitosis¹ or starvation²⁻⁴. Under these conditions a reversible inhibition of the initiation step of protein synthesis decreases the rate at which ribosomes become attached to messenger RNA. The continuing runoff of ribosomes from messenger during protein chain elongation and termination depletes the percentage of the total ribosomes which are in the form of polysomes and are synthesising protein.

Tetrahymena is a convenient cell in which translational control can be studied by measuring the percentage of polysomes under a variety of conditions. Of particular interest are the effects of temperature shifts which synchronise cell division in *Tetrahymena* cultures^{5,6} and which cause rapid and reversible inhibition of the initiation step in mammalian cells⁷⁻¹⁰. This report shows that temperature shifts, hypoxia and deprivation of nutrients all cause rapid, reversible decreases in the percentage of polysomes in *Tetrahymena* due to an inhibition of initiation. In previous reports decreases in the percentage of polysomes in this organism were noted only after long periods of cold shock¹¹ or starvation¹², and fractionation techniques were used which permitted considerable degradation of the polysomes.

Tetrahymena pyriformis (strain T) growing at 28°C in 2% proteose peptone-0.2% yeast extract was harvested in exponential phase and lysed with detergent in the presence of 100 μg heparin ml^{-1} . Centrifugation of the lysates on sucrose density gradients showed that about 75% of the

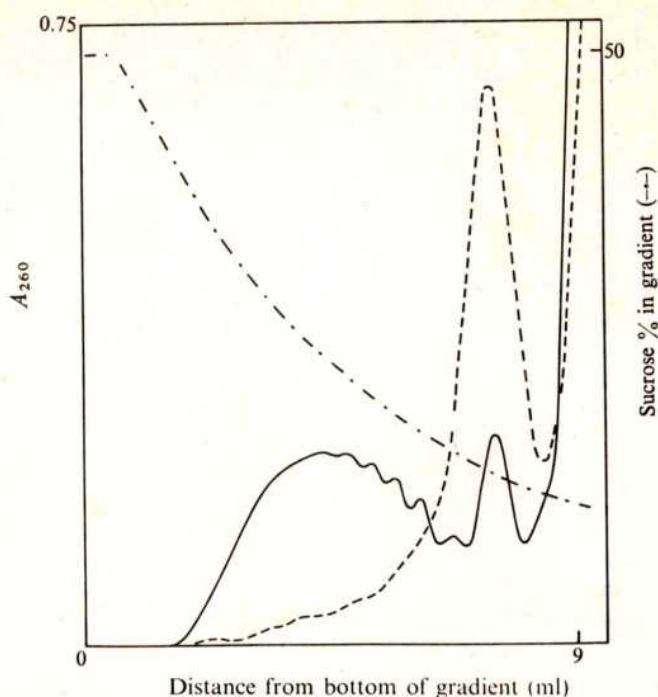


Fig. 1 Sucrose gradient centrifugation of *Tetrahymena* polysomes. Cells were collected by chilling the cultures ($0.5 - 1 \times 10^5$ cells ml^{-1}) and centrifuging at 0°C , washed once with 1 mM MgCl_2 - 10 mM KCl -10mM tris-HCl (pH 7.4) - 40 mM NaCl , suspended in 5 mM MgCl_2 - 5 mM mercaptoethanol - 20 mM Tris-HCl (pH 7.4) - 100 mM KCl - 250 mM sucrose containing 100 μg heparin ml^{-1} , and lysed by adding Nonidet-P40 to 0.2% and sodium deoxycholate to 1.0%. The lysate was centrifuged (12,000g, 15 min) and portions of the supernatant (equivalent to approximately 3×10^5 cells) were layered over non-isokinetic sucrose gradients¹³ (8.5 ml 10% to 50% sucrose over 0.5 ml 50% sucrose) containing 5 mM MgCl_2 - 10 mM Tris-HCl (pH 7.4) - 100 mM KCl . After centrifugation at 4°C (75 min, 30,000 r.p.m.) the distribution of ribosomes and polysomes was determined by pumping the gradient from the bottom of the tube through an LKB 4 mm square section flow cell. The extinction at 260 nm was continuously recorded with a Gilford model 2000 recording spectrophotometer. Treatment with pancreatic ribonuclease (25 μg ml^{-1} , 10 min at 25°) was carried out just before layering on the gradient. —, control; ---, RNase.

ribosomes were in the form of polysomes which sedimented more rapidly than the free (80S) ribosomes, but which were degraded to 80S structures by added pancreatic ribonuclease (Fig. 1).

The effects of inhibitors of translation demonstrated that the polysomes in the cell were steady-state structures resulting from a balance between the rate of ribosome attachment (initiation) and the rate of ribosome runoff. Cells incubated in growth medium containing ^{14}C -leucine (0.5 μCi ml^{-1} , 50 μCi μmol^{-1}) incorporated radioactivity into hot trichloroacetic acid-precipitable material at a linear rate for 60 min. This incorporation was inhibited 70% by 50 μM 2-(4-methyl-2,6-dinitroanilino)-N-methyl propionamide (MDMP), an inhibitor of initiation^{14,15}, and 50% by cycloheximide (0.5 μg ml^{-1}) which inhibits chain elongation. Figure 2 shows that 50 μM MDMP decreased the proportion of polysomes to 30% in 10 min. This effect was reversed by low concentrations of cycloheximide which, by partially inhibiting chain elongation, slow down the transit of ribosomes over the messenger, so preventing the depletion of the polysomes by the initiation inhibitor (see for example ref. 1). In the same way cycloheximide added to control cells increased the percentage of polysomes to 85-90%.

In contrast with these rapid effects other experiments (results not shown) demonstrated that 50 μg actinomycin ml^{-1} (which inhibited ^{14}C -uracil incorporation into the RNA

of intact cells by 95%) affected the percentage of polysomes much more gradually. Thus the percentage of polysomes fell from 76% to 70% after 30 min, and to 40% after 90 min, while ^{14}C -leucine incorporation was approximately 50% inhibited after 90 min. This suggests that rapid changes of the percentage of polysomes are unlikely to depend on changes in the rate of RNA synthesis.

When cells in growth medium were subjected to heat shock by raising the temperature to 34°C for 10 min the proportion of polysomes decreased to 45% (Fig. 3a and Table 1) and ^{14}C -leucine incorporation by the intact cells was 33% inhibited. When the cells were returned to 28°C , recovery was essentially complete and was unaffected by the presence of actinomycin (50 μg ml^{-1}). The decreased proportion of polysomes at 34°C reflected an altered balance between attachment and runoff of ribosomes, and cycloheximide (0.5 μg ml^{-1}) restored the percentage of polysomes to approximately the normal level presumably by slowing down the runoff of ribosomes (Table 1). A decrease in the number of polysomes could result from a decreased rate of ribosome attachment to mRNA (initiation) or from an increased transit rate of ribosomes over mRNA. Since protein synthesis was inhibited at 34°C the decreased percentage of polysomes probably reflected an inhibition of initiation. After 30 min at 34°C recovery at 28°C was incomplete (Table 1) which suggests a loss of messenger during the longer period at 34°C . It has been shown that at this temperature RNA in *Tetrahymena* breaks down more rapidly¹⁶ and stability of messenger (measured subsequently at 28°C as the capacity for protein synthesis in the presence of actinomycin) is decreased¹⁷.

Shifting the cells to 10°C inhibited protein synthesis by 80% and affected the polysomes in the same way as heat shock (Table 1 and Fig. 3b). Thus the percentage of polysomes was approximately 40% after 15 min or 60 min

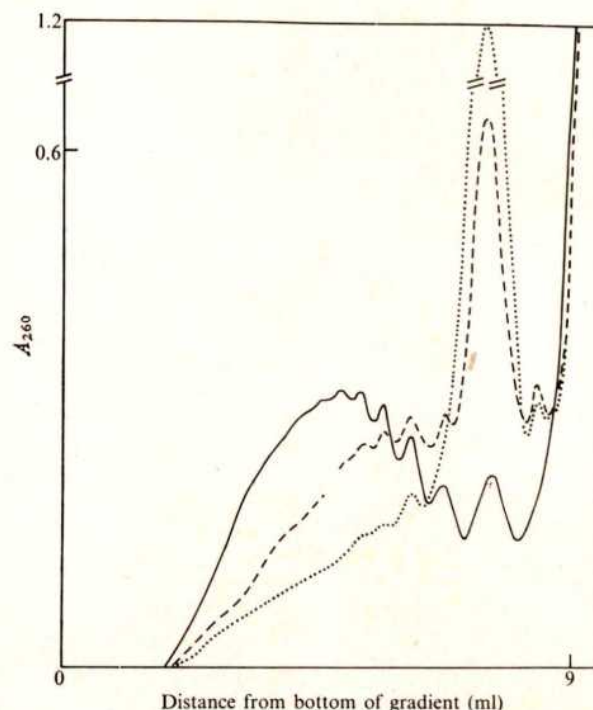


Fig. 2 Effect of MDMP (....), cycloheximide (—) and MDMP plus cycloheximide (---) on *Tetrahymena* (polysomes). Cell cultures in growth medium were incubated at 28°C for 10 min with added cycloheximide (0.5 μg ml^{-1}) or MDMP (50 μM). Samples of cell lysate (equivalent to approximately 5×10^5 cells) were centrifuged as in Fig. 1. Values for percentages of polysomes determined from the absorbancy tracing at 260 nm are cycloheximide 85%, MDMP 30%, MDMP + cycloheximide 61%. The values for controls without inhibitor were 72-76%.

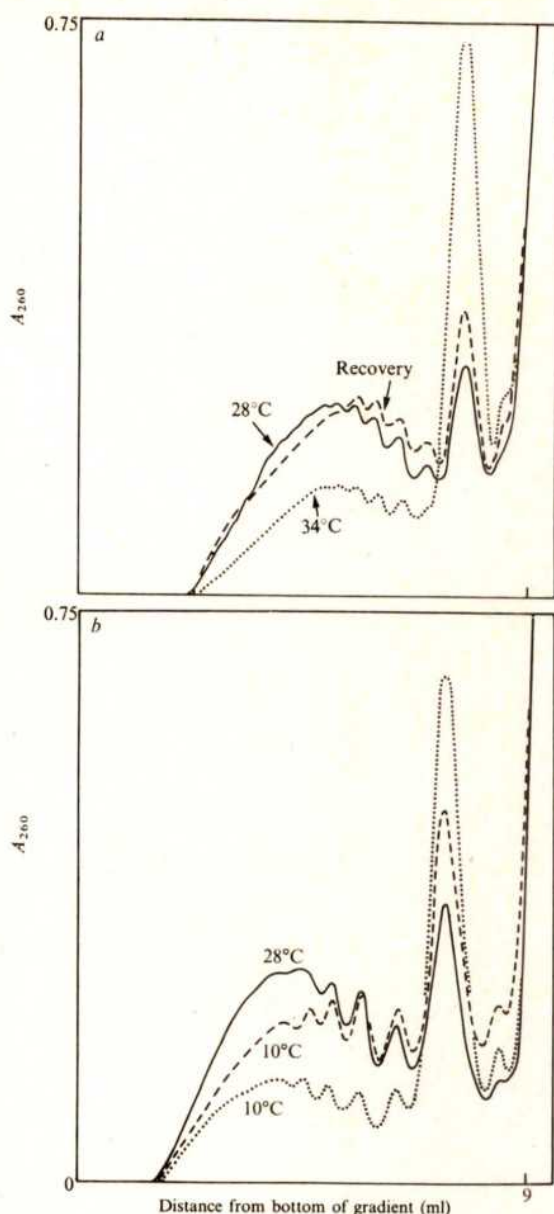


FIG. 3 Effect of temperature shifts on *Tetrahymena* polysomes. Cultures in growth medium were incubated as follows. *a*, (Heat shock): at 28°C, 10 min at 34°C, or 10 min at 34°C followed by recovery for 10 min at 28°C. *b*, (Cold shock): at 28°C, 15 min at 10°C, or 15 min at 10°C in the presence of cycloheximide ($0.5 \mu\text{g ml}^{-1}$) (— — —).

of cold shock and this decrease was prevented by cycloheximide. Recovery at 28°C was rapid and virtually complete even after 60 min at 10°C. This is in agreement with the finding that messenger is stable at low temperatures¹⁷.

Hypoxia has been used to synchronise *Tetrahymena*¹⁸ and may have an effect analogous to starvation of energy substrates⁴ which, like amino acid starvation^{3,4}, inhibits initiation of protein synthesis in mammalian cells. After 30 min of hypoxia in growth medium or 10 min of starvation in a saline medium the polysomes were decreased to about 40% (Table 1). In both cases cycloheximide prevented the depletion of polysomes and recovery was rapid though incomplete when normal conditions were restored. If the unlikely possibility that hypoxia or starvation increased the rate of ribosome transit on mRNA is excluded, then the decrease in the number of polysomes under these conditions must have resulted from a reversible inhibition of the initiation step.

Therefore, as in mammalian cells, temperature shifts and starvation seem to decrease the percentage of polysomes in

TABLE 1 Percentage of Polysomes in *Tetrahymena* under Various Conditions

Incubation conditions	% of polysomes		
	Incubation without cycloheximide	Incubation with cycloheximide	Incubation without cycloheximide, then 10 min at 28°C in growth medium
34°C for 10 min	45	78	74*
34°C for 30 min	46	—	62
10°C for 15 min	41	71	—
10°C for 60 min	40	—	71*
Hypoxia, 30 min	42	73	64
Starved, 10 min	42	60	64†

The percentage of polysomes was determined as in Fig. 2 after temperature shift (Fig. 3), hypoxia (cultures in growth medium at 28°C gassed with nitrogen) or starvation (cells suspended in 1 mM MgSO_4 — 5 mM potassium phosphate (pH 7.0) — 45 mM NaCl at 28°C). These conditions were modified as shown by including cycloheximide ($0.5 \mu\text{g ml}^{-1}$) or by permitting recovery by subsequent incubation for 10 min in growth medium at 28°C. Values are averages of two or more results (which agreed within approximately 5%). In control cells (growth medium at 28°C) the proportion of polysomes was 73–77%.

* Results after recovery in the presence of actinomycin ($50 \mu\text{g ml}^{-1}$) were essentially the same.

† Similar results were obtained when an equal volume of double-strength growth medium was added or when the cells were centrifuged at 0°C and resuspended in growth medium at 28°C.

Tetrahymena by inhibiting initiation. Messenger not engaged in polysome formation may as in *Escherichia coli*^{19,20} be more susceptible to degradation, especially at higher temperatures. In this way inhibition of initiation during relatively short periods at 34°C or longer periods at 10°C could contribute to that depletion of messenger for proteins related to cell division which is the probable basis of synchronisation⁵.

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New genetic and physicochemical data on structure of dinoflagellate chromosomes

THE majority of free-living dinoflagellates differ from other eukaryotes in possessing high chromosome numbers¹, large amounts of DNA² per cell, uniform chromosome size and in their apparent lack of histone proteins³. One of their most interesting properties which they share along with the euglenids⁴ is the condensed state of their chromosomes throughout the cell cycle⁵.

These chromosome properties immediately suggest three simple models for nuclear DNA organisation: (1) a normal pattern of alternation of haploid and diploid generations with each haploid chromosome non-identical; (2) polyploidy, having three or more sets of homologous chromosomes and (3) polyteny, a multistranded condition of chromosomes found for example in *Drosophila* salivary glands. Haapala and Soyer⁶ have recently proposed a diploid-polyteny model for the organisation of the dinoflagellate nucleus. On the basis of our work on *Cryptocodinium cohnii* (Seligo) Chatton in Grassé, 1952 (GC strain, Indiana University Culture Collection No. 1649), and other dinoflagellates we take issue with this model for the following reasons. Haapala and Soyer⁶ assumed dinoflagellates to be diploid. To date all except possibly *Noctiluca*⁷ have been cytologically determined to be haploid⁸⁻¹². Our chemical mutagenesis studies using N-methyl-N'-nitro-N-nitrosoguanidine have demonstrated that nutritional and carotenoid deficient mutants can be obtained at a frequency similar to bacteria¹³. (Chemical mutagenesis of *C. cohnii* was carried out by the method of Adelberg *et al.*¹³, with the following modifications: temperature = 25° C, final concentration of nitrosoguanidine = 20 µg ml⁻¹, carried out in M buffer (342 mM NaCl, 28 mM MgSO₄, 2 mM CaCl₂, 9 mM KCl, 8 mM 4-morpholine-ethane sulphonic acid, final pH = 6.0). Treatment time = 6 min. Under these conditions, the mutation frequency for purine and pyrimidine requiring strains was in the range of 0.17-0.83% of survivors and for carotenoid deficient strains in the range of 0.2-0.29% of survivors. Adelberg *et al.*¹³ obtained valine resistance in *E. coli* K12 at a maximum frequency of 0.20% survivors.) Only a haploid nuclear condition would be consistent with the frequency of mutations obtained. To find a viable mutant of diploid organism one would have to mutate the same gene on both homologous chromosomes without causing additional lethal mutations elsewhere in the genome. So, the frequency of obtaining conditionally lethal mutants is very small in a diploid organism. For the above reasons mutational data also rule out simple polyteny and polyploidy, where every single gene on every chromosome is polytenised or where every chromosome is present in two or more copies. Our DNA renaturation kinetic studies on *C. cohnii* also rule out polyteny and extreme polyploidy, where every chromosome is exactly the same. From DNA measurements on exponentially growing cells we find 6.9 µg per 10⁶ cells by two spectrofluorometric methods: ethidium bromide¹⁴, and diaminobenzoic acid^{15,16}, which agree with the reported value of 6.9 µg per 10⁶ cells obtained with the diphenylamine method¹⁷. Using the acetocarmine technique¹⁸ we have obtained counts of 107 and

110 chromosomes for *C. cohnii* in close agreement to the reported¹⁹ value of 99. Assuming all of the chromosomes have roughly the same amount of DNA, we calculate 4.2×10^{10} daltons per chromosome. If extreme polyploidy were the case, then the slowest renaturing component of *C. cohnii* DNA would renature at a rate no more than twenty times slower than *Escherichia coli* strain C600 DNA which has about 2.2×10^9 daltons per chromosome¹⁴. Figure 1 presents renaturation kinetic measurements of *C. cohnii* and *E. coli* DNA. Although it is obvious that there is a component comprising roughly 60% of the dinoflagellate DNA which renatures at a rate comparable to *E. coli* the remaining 40% is not renatured at C_0t value sixty times greater than the $C_0t_{1/2}$ of

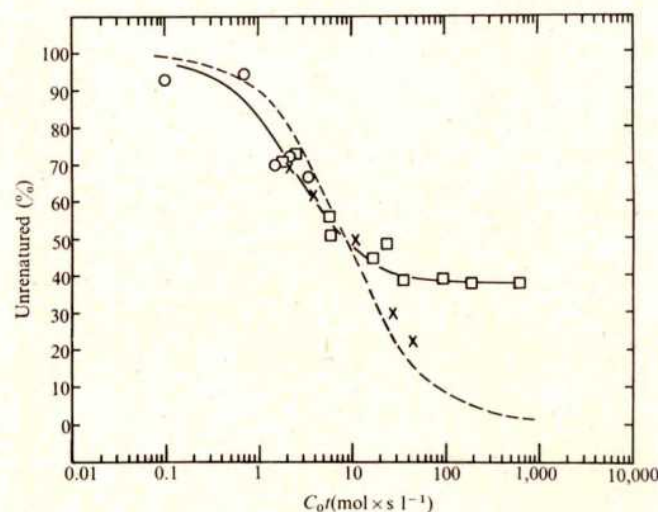


FIG. 1 The kinetics of re-association of the DNA from the dinoflagellate *C. cohnii* compared with that of the bacterium *E. coli* C600. The DNA of both organisms was isolated by the method of Marmur²⁰ and tested for purity by CsCl equilibrium density ultracentrifugation and optical melting analysis. DNA was sheared by sonication to fragments of 500 ± 50 nucleotide pairs (as measured by sedimentation rate in the Spinco Model E ultracentrifuge). Samples of DNA in 120 mM phosphate buffer were denatured by heating to 100° C for 5 min and then allowed to renature for various times at $60 \pm 1^\circ$ C. The samples were then passed over a hydroxyapatite column at 64° C to separate quantitatively single and double stranded DNA. DNA concentrations were: \times , 190 µg ml⁻¹ *E. coli* DNA; \square , 500 µg ml⁻¹ *C. cohnii* DNA; \circ , 100 µg ml⁻¹ *C. cohnii* DNA.

E. coli DNA. If *C. cohnii* were 500 times polytene as proposed by Haapala and Soyer⁶, the slowest component of the DNA should renature no more than four times more slowly than *E. coli* DNA; that is, $C_0t_{1/2} \leq 40$. Our measurements indicate the $C_0t_{1/2}$ of the 60% component of the dinoflagellate is about 1, and its kinetic complexity is estimated to be 2.5×10^8 daltons ($1/10 \times$ kinetic complexity of *E. coli* DNA). The amount of DNA comprising this component is 2.4×10^{12} daltons per nucleus, thus the sequences are repeated about 10,000 times. Repeated sequences of such high complexity are unusual, and might be important in establishing the evolutionary history of dinoflagellates. The remaining 40% of the DNA is not appreciably renatured at C_0t 600. Since the C_0t plot representation of the renaturation of the 40% component must have a finite slope no steeper than -20 (% unrenatured/ $\log C_0t$) as predicted from theory, the $C_0t_{1/2}$ of this slowly-renaturing component can be no less than 3000; and thus the DNA of this component can be repeated no more than ten times per cell. Thus our renaturation kinetics and chemical mutagenesis studies do not support the conclusions of Haapala and Soyer⁶ of a diploid-polytene nature of the dinoflagellate nucleus. Their suggestion of multiple circular DNA molecules composing a dinoflagellate chromosome is

however, very intriguing. Whether each chromosome contains one or several pieces of DNA may be tested using the viscoelastic technique^{14,21}.

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Protein densities from X-ray crystallographic coordinates

DENSITIES of the interiors of nine protein molecules were calculated using atomic coordinates obtained by X-ray crystallography. The resulting values, obtained for spherical regions of the molecules surrounding their centres of mass, are 10–25% below the apparent densities of the same molecules in solution. These regions include 20–50% of the total molecular mass. The densities calculated were 1.09–1.25 g cm⁻³ whereas the observed apparent solution densities of these proteins are 1.33–1.42 g cm⁻³.

Calculations were performed as follows. All atoms in each molecule were identified and assigned appropriate masses, including hydrogens, which are not generally located by protein X-ray crystallography (thus, all α -carbons except proline were given mass 13; all peptide nitrogens except proline, 15; the alanine β -carbon, 15; the lysine epsilon amino nitrogen, 17, and so on). The molecular centre of mass was located and its distance from each atom was determined. The masses of all atoms within shells 0–2 Å, 2–4 Å, 4–6 Å, and so on, from the centre of mass were added, and the sum

divided by the shell volume to give a 'shell density.' 'Mean densities' for spheres of radii 2 Å, 4 Å, 6 Å, and so on, were also calculated by dividing the total mass enclosed within each sphere by the sphere volume.

Results for five proteins are shown in Figs 1 and 2. The innermost shell densities vary widely due to their few atoms. Farther out in the molecule shell and mean densities of 1.02–1.27 and 1.14–1.22 g cm⁻³, respectively, are found. When shells become large enough to include solvent molecules (which were not included in the calculations), the shell density declines. The outer radius of the shell beyond which this decline begins is called R_{max} ; all points within R_{max} are presumed to lie entirely within the molecular cavity. The

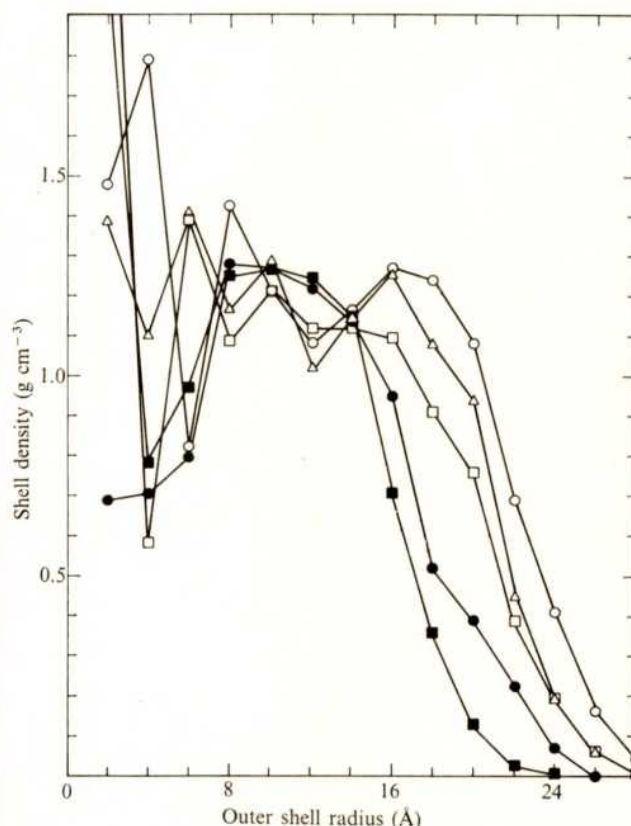


FIG. 1 Dependence of shell densities on shell radius. ○, Carboxypeptidase; △, subtilisin; □, chymotrypsin; ●, myoglobin; ■, lysozyme.

mean density of the sphere of radius R_{max} , calculated as just described, is denoted by ρ_{calc} , and the observed apparent density of the protein, as obtained from solution density measurements, is ρ_{obs} . (In two instances ρ_{obs} will be the value calculated by the Cohn-Edsall method, which generally agrees well with solution density values¹.)

Table 1 gives R_{max} , ρ_{calc} and ρ_{obs} for nine proteins. Column 6 shows that ρ_{calc} is 0.10–0.36 g cm⁻³ below the corresponding ρ_{obs} (the mean difference is 0.21). Column 3 gives the fraction of the total molecular mass within the sphere of radius R_{max} . The abnormally low densities refer to only the central 20–50% of the molecule.

Three explanations for this discrepancy are conceivable. (The possibility that the apparent protein density in the crystal differs to this extent from the density in solution seems to be excluded by the measurements of Low and Richards^{2,3} and of McMeekin, Groves and Hipp⁴.)

(a) The protein has the density ρ_{calc} throughout, but may be surrounded by an abnormally dense water jacket. To estimate the density in this jacket an equivalent radius, R_{eq} , was calculated for two of the proteins from the known

TABLE 1 Density calculations for nine proteins

(1) Protein	(2) R_{\max} (Å)	(3) Mass fract.	(4) ρ_{calc} g cm ⁻³	(5) ρ_{obs} g cm ⁻³	(6) $\rho_{\text{obs}} - \rho_{\text{calc}}$	(7) n_+ (ref. 7)	(8) n_- (ref. 7)	(9) $\Delta\rho_{\text{elec}}$ g cm ⁻³	(10) ρ_{outer} g cm ⁻³	(11) $\rho_{\text{outer}} - \rho_{\text{calc}}$	(12) $n_{\text{H}_2\text{O}}$
Myoglobin*	12	0.291	1.19	1.35 [§]	0.16	23	22	0.046	1.43	0.24	117
Lamprey haemoglobin*	14	0.477	1.15	1.34 [§]	0.19	18	24	0.042	1.56	0.41	128
Carboxypeptidase	18	0.527	1.23	{1.33 [†] 1.39 [‡]	{0.10 0.16	26	26	0.029	{1.47 1.63	{0.24 0.40	{148 240}
Chymotrypsin	14	0.320	1.14	1.37 [‡]	0.23	17	14	0.024	1.51	0.37	228
Chymotrypsinogen	14	0.294	1.09	1.38 [§]	0.29	18	14	0.024	1.56	0.47	304
Lysozyme	12	0.381	1.25	1.42 [‡]	0.17	17	10	0.038	1.55	0.30	96
Ribonuclease	10	0.194	1.05	1.41 [‡]	0.36	14	10	0.035	1.54	0.49	195
Staphylococcal nuclease	12	0.325	1.20	1.39 [‡]	0.19	28	19	0.056	1.51	0.31	122
Subtilisin	16	0.447	1.18	1.38 [‡]	0.19	23	25	0.033	1.58	0.39	209

* These include contributions of haem groups.

† An assumed value, included to show, by comparison with the alternate value, the sensitivity of columns (10)–(12) to the value of ρ_{obs} .

‡ Same value is obtained when ρ is calculated from the amino acid composition by the Cohn-Edsall method¹.

§ Value calculated from amino acid composition by the Cohn-Edsall method¹.

TABLE 2 Density of water jacket required to raise the calculated densities, ρ_{calc} , to the observed apparent solution densities, ρ_{obs}

Jacket thickness (Å)	3.0	6.0	9.0	15.0
ρ_{jacket} { Myoglobin g cm ⁻³ { Carboxypeptidase	{ 1.624 1.547	{ 1.467 1.426	{ 1.418 1.387	{ 1.381 1.357

total molecular mass and ρ_{calc} . ($R_{\text{eq}} = (3M/4\pi N_0 \rho_{\text{calc}})^{1/3} = 18.08$ Å for myoglobin, with $M = 17756$, $\rho_{\text{calc}} = 1.19$; $R_{\text{eq}} = 22.28$ Å for carboxypeptidase, with $M = 34337$, $\rho_{\text{calc}} = 1.23$.) The required jacket density is then $\rho_{\text{jacket}} = [\rho_{\text{obs}}(R_{\text{eq}} + t)^3 - \rho_{\text{calc}}R_{\text{eq}}^3]/[(R_{\text{eq}} + t)^3 - R_{\text{eq}}^3]$, t being the jacket thickness. The value $t = 3.0$ Å is equivalent to a jacket about one water molecule thick. Table 2 shows that water densities of 1.35–1.60 are required to bring ρ_{calc} to the observed value if the jacket is 1–5 water molecules thick. (ρ_{obs} was given values 1.35 for myoglobin and 1.33 for carboxypeptidase.)

Electrostriction by charged groups could account for only a portion of such a density increase. Columns 7, 8 and 9 of Table 1 give the numbers of positively and negatively charged side chains on each protein molecule and the apparent density increase, $\Delta\rho_{\text{elect}}$, resulting from a 10 cm³ mol⁻¹ electrostrictive contraction per charged group. Evidently $\Delta\rho_{\text{elect}}$ is 0.025–0.05 whereas the discrepancy, $\rho_{\text{obs}} - \rho_{\text{calc}}$, is 3.4–12 times greater than this.

A structureless water jacket with $\rho = 1.3$ –1.6 might be detectable as a halo of high electron density on X-ray Fourier maps; we understand that this is not observed.

(b) The outer portion of the molecule may have a higher density than the central region. This difference would be expected if the interior of the molecule contains mostly hydrophobic groups and if polar groups tend to be found near the molecular surface. (Solid straight chain paraffins and benzene have densities of about 1.0. Most proteins, however, have peptide groups buried in their interiors as β -sheets and α -helices. Cohn and Edsall give 20 cm³ as

the mole volume of the $-\text{CO}-\text{NH}-$ group, equivalent to 2.15 g cm⁻³. Thus the interior density should be somewhat above 1.0.) Apparent densities, ρ_{outer} , of the outer regions were calculated by dividing the difference between the total molecular weight and the mass enclosed within R_{max} by the difference between the volume bounded by R_{max} and the total molecular volume, as calculated from the molecular weight and the observed partial specific volume in solution. These are shown in Table 1, column 10. Values range from 1.43 to 1.63; all but two are above 1.50. These densities are large compared with the known densities of solid peptides. Among the solid simple peptides only diglycine (1.71) and glycyl alanine (1.55) have such high densities. Solid polyglycine has a density of 1.51, but other solid synthetic polypeptides are much less dense: polyalanine, 1.28; poly γ -methyl L-glutamate, 1.32; poly γ -benzyl DL-glutamate, 1.28. Silk fibroin, a rather polar extended protein molecule, has a density of 1.36. Densities of about 1.50–1.55 for the molecules' outer regions are thus somewhat above what might be expected from the known densities of peptides, polypeptides, and dry proteins generally.

The polar and non-polar character of regions inside and outside R_{max} are compared for four proteins in Table 3, which shows the numbers of different types of atoms with distances from the centre of mass less than and greater than R_{max} . In these proteins, hydrophobic atoms are slightly more common in the interior than in the exterior. None of the $\phi_</\phi_>$ values can, however, be considered to indicate a tendency for the typical protein molecule interior to be markedly more hydrophobic than the exterior—a fact already evident from the work of Lee and Richards⁵. It is thus difficult to attribute the density discrepancy to a markedly different composition of the inner and outer portions of these protein molecules.

(c) Many water molecules may exist throughout the interiors of protein molecules. Column 12 of Table 1 shows the number of internal water molecules required to account for the difference between ρ_{calc} and ρ_{obs} . Numbers of the

TABLE 3 Numbers of different types of atoms present inside and outside of a sphere of radius R_{max} centred on the centre of mass

Protein	$R < R_{\text{max}}$				$R > R_{\text{max}}$				$\phi_<$	$\phi_>$	$\phi_</\phi_>$
	C_α	C_{hyd}	Polar atoms	Haem	C_α	C_{hyd}	Polar atoms	Haem			
Lysozyme	49	144	178	0	79	211	339	0	0.809	0.622	1.30
Myoglobin	48	173	148	34	108	334	446	10	1.399	0.771	1.81
Carboxypeptidase	162	538	583	0	145	409	599	0	0.923	0.682	1.35
Subtilisin	131	295	439	0	144	359	575	0	0.672	0.624	1.08

'Polar atoms' include oxygen, nitrogen and carbonyl carbons, ' C_α ' are the main chain α -carbon atoms, and ' C_{hyd} ' are all other carbon atoms—considered to be hydrophobic. $\phi_<$ and $\phi_>$ are the ratios of the numbers of ' C_{hyd} ' + 'Haem' atoms to 'polar atoms' for $R < R_{\text{max}}$ and $R > R_{\text{max}}$, respectively.

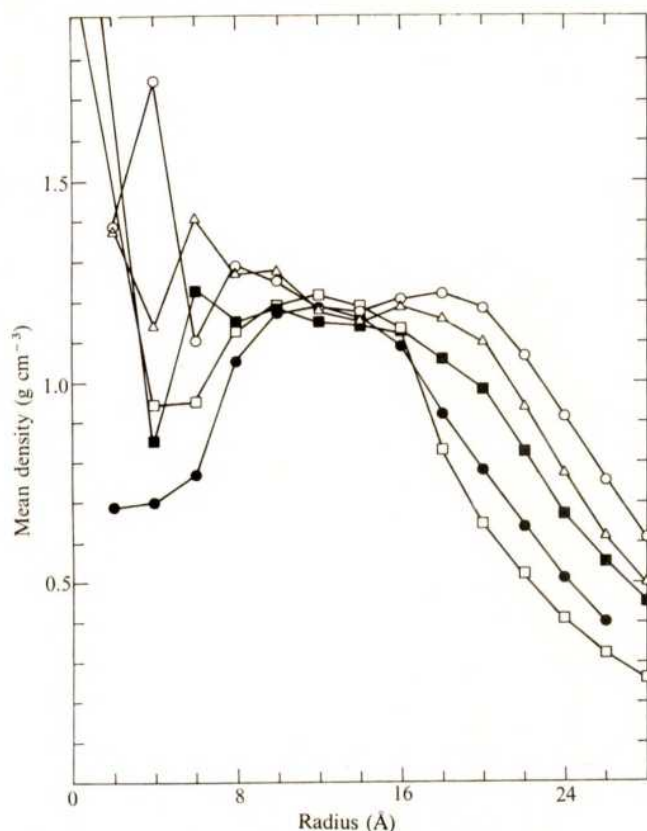


Fig. 2 Dependence of mean density of spherical regions on sphere radius. \circ , carboxypeptidase; Δ , subtilisin; \blacksquare , chymotrypsin; \bullet , myoglobin; \square , lysozyme.

order of 100–300 are indicated. Birktoft and Blow⁶ have detected approximately 16 localised water molecules within the chymotrypsin molecule. A much larger number than this is believed unlikely for proteins in general.

The second explanation (low density centre, higher density exterior) seems to be the most plausible, but contributions from all three factors are conceivable.

Atomic coordinates used in this study were supplied by the following: carboxypeptidase, W. Lipscomb; chymotrypsin, D. M. Blow; chymotrypsinogen, H. T. Wright; subtilisin, J. Kraut; lamprey haemoglobin, W. Love; lysozyme, D. Phillips; myoglobin, J. C. Kendrew; ribonuclease, F. M. Richards; staphylococcal nuclease, F. A. Cotton. The Princeton University Computer Centre facilities were used in making the calculations. The work was supported by a US National Science Foundation grant.

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Control of Zea root elongation by light and the action of 3,5-diiodo-4-hydroxybenzoic acid

THE inhibitory effect of white light on the elongation of the primary roots of wheat and rice seedlings has been reported^{1–4}. It would seem that light affects the ability of the cells to increase in length and also reduces the meristematic activity of the root^{1,4}; the latter process may be dependent on the wavelength of the incident radiation⁴. Other studies here have shown that the chemical 3,5-diiodo-4-hydroxybenzoic acid (DIHB) can completely remove the inhibitory effect of light on the roots of rice and cress seedlings. This was achieved mainly by enhancement of cell elongation and not by stimulation of cell multiplication^{5,6}.

Following the discovery, in these laboratories, of the plant growth inhibitor xanthoxin (formed as a photo-oxidation product of certain xanthophyll epoxides⁷ known to occur in cereal roots^{8,9}) we decided to assess the possible role of the new inhibitor in the control of root elongation by light in relation to the action of DIHB.

Initial investigations were carried out to establish whether an exogenous application of xanthoxin affected the elongation of the primary roots of *Triticum* seedlings. Eclipse wheat seeds germinated for 20 h at 25°C in darkness to give a radicle length of 1 mm, were placed on Whatman No. 1 filter paper (5.5 cm diameter) in glass Petri dishes containing 2 ml of the appropriate test solution. Root length was measured after a 20 h incubation period at 25°C in white light (Mazda daylight fluorescent tube) or total darkness. Light

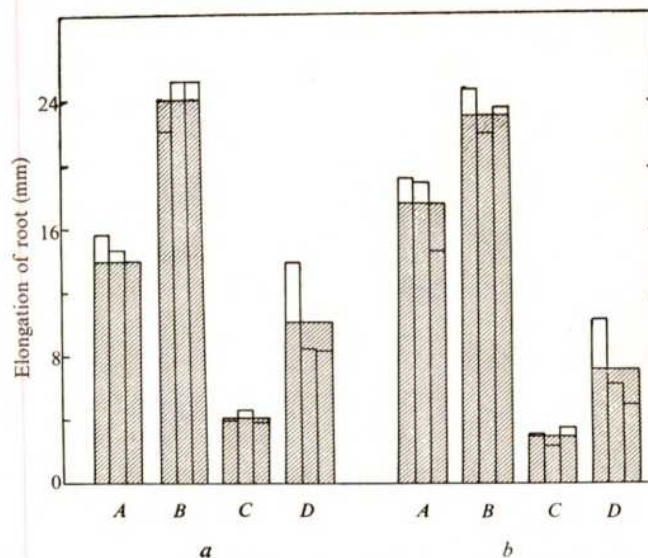


Fig. 1 Effect of 10 μ M xanthoxin and 0.1 mM DIHB solutions on the elongation of the primary roots of *Triticum* seedlings. Shaded areas are the mean of three independent experiments in which each treatment consisted of eight replicate seedlings. A, Control; B, DIHB; C, xanthoxin; D, xanthoxin and DIHB. a, light; b, dark.

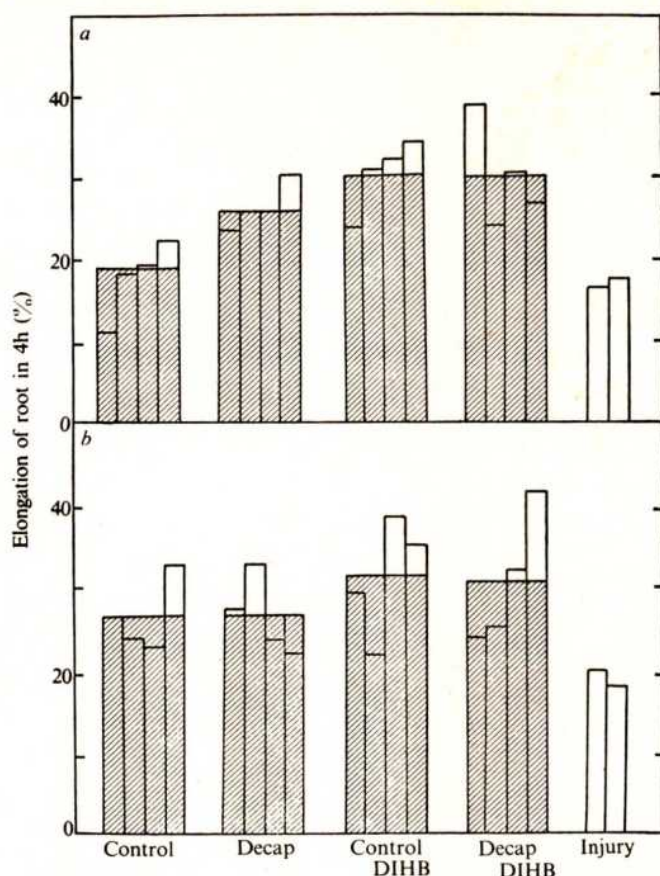


FIG. 2 Effect of root decapitation and 0.1 mM DIHB solution on the elongation of the roots of *Zea* seedlings exposed to white light or darkness. Shaded areas are the mean of four independent experiments in which each treatment consisted of eight replicate seedlings. a, light; b, dark.

exposure was found to induce a 21% reduction in the length of the wheat seedling roots. Application of a 10 μ M aqueous solution of xanthoxin resulted in an 83% reduction in elongation in the dark and a 72% reduction in the light compared with the water controls, (Fig. 1). Treatment with 0.1 mM DIHB solution gave a 56% increase in root elongation in the light and a 25% increase in darkness. A solution of 10 μ M xanthoxin, together with 0.1 mM DIHB, however, caused a 59% reduction of root elongation in the dark and only a 27% reduction in the light. In other words, DIHB seems to antagonise the inhibitory effect of xanthoxin on root elongation and this antagonism is more pronounced in the light than in the dark. An interaction of this type would be expected if xanthoxin was, in fact, involved in the endogenous inhibition of root elongation.

Further investigation⁹ revealed that xanthoxin could not be isolated from the roots of *Zea* seedlings 2-d-old exposed to white light. This finding was somewhat surprising as violaxanthin and neoxanthin were shown to be present in the light-exposed roots. The possibility still exists, however, that the rate of utilisation of the inhibitor might be so rapid that isolation of significant amounts would be difficult.

Microsurgical removal of the root cap of *Zea* has been shown to prevent the response of the roots to gravity¹⁰ and we have found that this treatment completely releases the roots from the inhibitory effect of light on elongation⁹. We decided, therefore, to determine whether the root growth-stimulatory properties of DIHB in the light were associated with this region of the root. Seedlings of *Zea mays* var. LG 11, 3-d-old and grown in darkness at 25° C on moistened Whatman No. 1 filter paper, were selected in green light for straight roots 20–30 mm long. Tests were carried out in glass Petri dishes 9 cm in diameter containing filter paper and

7 ml of the appropriate solution. Root length was measured immediately following treatment and again after a 4 h incubation period in white light or total darkness. Decapitated and control seedlings (with intact root apices) were placed in contact with distilled water or 0.1 mM DIHB solution. An additional control in which a 1 mm deep vertical incision was made into the root cap was used to establish the effect of injuring the root apex.

Roots of the water control increased in length by 18% and 27% during the 4 h incubation period in white light and darkness respectively, revealing again the inhibitory effect of light (Fig. 2). Decapitation of the roots of the seedlings exposed to white light resulted in an elongation equal to that of dark controls with intact apices⁹. Decapitation of the roots of the darkened seedlings, however, seemed to have no effect on root elongation. As the treatment designed to show the effect of injury to the root apex did not produce an enhancement of elongation, it was concluded that the root cap of *Zea* seedlings is implicated in the light-induced control of root elongation.

Application of DIHB to control and root-decapitated seedlings in the light and dark gave a uniform elongation of 31% to 32% during the 4 h incubation period. It is therefore clear that in presence of DIHB the decapitation had little effect on root elongation. Thus, it seems that the root growth-stimulatory properties of DIHB are independent of the light-induced control system which we have shown to be present in the root cap.

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Impact of cool temperatures on transformation of human and armadillo lymphocytes (*Dasypus novemcinctus*, Linn.) as related to leprosy

A CENTURY-LONG search for an unaltered animal in which to study leprosy in humans culminated in the finding that the nine-banded armadillo (*Dasypus novemcinctus*, Linn.), a primitive mammal native to the southern Western Hemisphere, develops disseminated lepromatous leprosy following inoculation with *Mycobacterium leprae* isolated from human tissue^{1–3}. Previous animals only developed leprosy following inoculation with leprosy bacilli after having been immunologically suppressed by neonatal thymectomy with or without irradiation of bone marrow^{4–8}. Yet curiously the leprosy lesions occurred chiefly on the cooler tissues, the footpads.

TABLE 1 Transformation of lymphocytes from 27 normal uninoculated armadillos to PHA at various temperatures.

	37° C		33° C		28° C	
	PHA	CT*	PHA	CT	PHA	CT
Mean	6,970	755	2,402	677	1,351	745
Standard deviation	1,908	196	1,707	196	491	178
Standard error of mean	367	38	329	38	263	34

Results expressed as an average value of duplicate cultures in c.p.m. of ^3H -thymidine taken up by lymphoblasts.

* CT, control, no PHA added.

Binford and Brand noted that humans with leprosy were most severely infected in the cooler regions of their bodies such as the nose and digits, and so suggested that *M. leprae* may grow better at low temperatures^{9,10}. Storrs first suggested that the armadillo be used for the study of leprosy because its low body temperature (30° C to 35° C) was comparable with the cool regions of humans¹.

We reasoned that such a cool body temperature might depress metabolic activity and from this, cellular immunity of the armadillo might also be depressed and so enable infection by *M. leprae*. To test this we investigated the effect of temperature on the transformation of lymphocytes from armadillos when stimulated by phytohaemagglutinin (PHA) and lepromin.

The transformation of lymphocytes *in vitro* by mitogens is considered a measure of cellular immunity of the individual. More specifically, transformation of lymphocytes by mitogens may reflect the capacity of an individual to respond to and to control certain infectious agents, mycobacteria, brucellae, fungi and viruses, and to reject allografts and neoplasms.

Initially, lymphocytes from 27 normal, uninoculated armadillos were cultured with PHA following procedures previously described¹¹, with the exception that 10% foetal calf serum was used in place of autologous plasma. Armadillos were anaesthetised with ketamine hydrochloride (50 mg kg⁻¹) and 10–15 ml of blood were drawn by intracardiac puncture into heparinised syringes and transferred into siliconised tubes. Lymphocytes were separated and divided into nine equal samples and cultured in triplicate. PHA was added to two of the cultures in each triplicate set and no PHA was added to the third culture which served as a control. One of each triplicate set was cultured at 37° C, the second at 33° C and the third at 28° C, in a humidified atmosphere of 5% CO₂ for 72 h. Lymphocyte transformation was measured by the uptake of ^3H -thymidine. A Packard Tricarb Scintillation Counter measured the results which were expressed as an average of duplicate samples in c.p.m.

The transformation of lymphocytes stimulated by PHA was reduced by approximately 66% when the lymphocytes were cultured at 33° C and by 81% when cultured at 28° C as compared with transformation of lymphocytes at 37° C (Table 1). Thus, a depression of lymphocyte responsiveness to PHA occurred at low temperatures or temperatures comparable with the body temperature of the armadillo.

Second, transformation of lymphocytes by lepromin was measured using lymphocytes from 13 armadillos. We used 0.1 ml of lepromin (containing 3×10^7 sonicated *M. leprae* ml⁻¹) as the specific mitogen, instead of the non-specific mitogen, PHA. The technique was otherwise identical to that used with PHA. Four of the 13 armadillos were uninoculated animals and seven were uninfected, but inoculated with *M. leprae* 30 months before the *in vitro* challenge of lymphocytes by lepromin. The remaining two armadillos which were inoculated with *M. leprae* were grossly infected with leprosy.

A marked reduction of transformation of lymphocytes by lepromin from all armadillos occurred at 33° C and 28° C when compared with transformation at 37° C. Lymphocytes

from uninoculated armadillos which were cultured at 37° C transformed at 5,621 c.p.m. (average) while the lymphocytes from armadillos inoculated but not grossly infected with *M. leprae* were more responsive, transforming at 14,505 c.p.m. (average). This increase in transformation of lymphocytes by lepromin reflects sensitisation of the lymphocytes resulting from inoculation with *M. leprae* 30 months previously. Furthermore, lymphocytes from the two inoculated armadillos with gross evidence of leprosy responded nearly identically to the uninoculated animals. The lymphocytes from the grossly infected armadillos averaged 5,680 c.p.m. This lack of an increase in the transformation response of lymphocytes from infected armadillos following stimulation by lepromin could be explained in several ways.

One possibility is the two infected armadillos were not sensitised following exposure to antigens which were acquired by inoculation with *M. leprae* and that the lack of sensitisation reflected the lack of resistance to *M. leprae* which led to lepromatous leprosy.

A second possibility is that the two infected armadillos were initially sensitised as a result of inoculation of *M. leprae* but later anergy developed similar to that occurring in humans with advanced lepromatous leprosy¹², due to a replacement of lymphoid tissues by invading *M. leprae*. But we are unable to explain why the lymphoblastic transformation response was comparable with that of normal armadillos and not more depressed.

Transformation of lymphocytes from humans was also studied to determine if cool temperatures would depress the transformation of lymphocytes. Heparinised blood (15 ml) was obtained by venepuncture in siliconised test tubes from nine healthy, young adult males and one woman. Three of the male subjects had been exposed to *M. leprae* while working for several years in an infectious diseases laboratory and the woman had worked for 5 yr in a

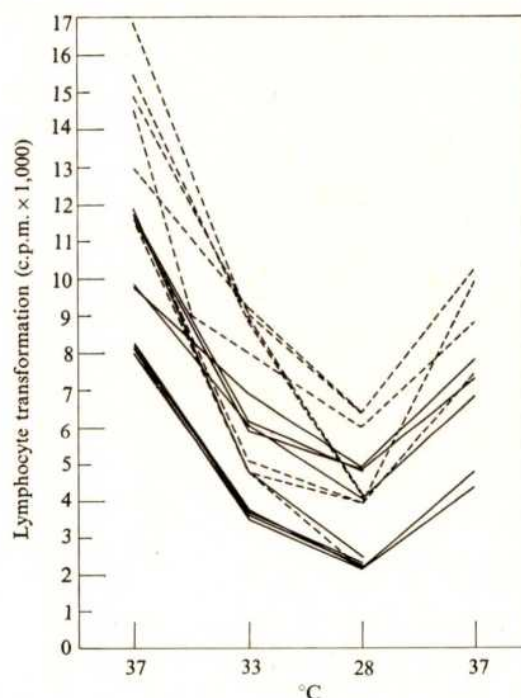


Fig. 1 The lymphoblastic transformation response of lymphocytes cultured at 37° C, 33° C, and 28° C when stimulated by PHA or lepromin is illustrated. An increase in transformation of lymphocytes is demonstrated to the right. The increase occurred when the lymphocytes were transferred from a 28° C incubator to a 37° C incubator and cultured for an additional 48 h. The c.p.m. is a measure of ^3H -thymidine taken up by transforming lymphoblasts. The average value of the control (no PHA or lepromin) was 998 c.p.m. —, Phytohaemagglutinin; ---, lepromin.

leprosum. The lymphocytes were cultured and stimulated by PHA and lepromin as had been done with lymphocytes from armadillos.

In all instances a depression of transformation of lymphocytes occurred at 33° C and 28° C (Fig. 1). The average value of the control, to which no mitogen was added, was 998 c.p.m. No increase in transformation by lepromin occurred in cultures of lymphocytes from the four human subjects who were previously exposed to *M. leprae* as compared with transformation of lymphocytes from six humans not exposed to *M. leprae*. This finding probably reflects non-sensitising exposure to antigens of *M. leprae* rather than an inability of the subject to become sensitised.

Lymphocytes which had been cultured for 72 h with PHA or lepromin at 28° C were transferred to an incubator at 37° C and cultured for an additional 48 h to determine whether the transformation response could be restored. The transformation response was restored by more than 50% (Fig. 1), thus directly implicating temperature as the agent responsible for the depression of the lymphocyte transformation.

We conclude that cool temperatures probably depress cell-mediated immunity *in vivo* and that such a depression of CMI could explain the susceptibility of armadillos to infection with *M. leprae*. Yet another possibility is that *M. leprae* multiplies best at cooler temperatures because a cool environment is optimal for their multiplication.

But, evidence to the contrary has been recently reported: more extensive multiplication of *M. leprae* *in vitro* has been reported in human macrophages at 37° C than at 33° C (ref. 13). If the optimal temperature for the growth of *M. leprae* is 37° C, then the armadillo's susceptibility to leprosy cannot be explained on this basis because its body temperature is much cooler than 37° C. Our finding of normal appearing thymolymphatic organs in the armadillo further supports that the cool body temperature of the armadillo is probably the significant condition responsible for the immune incompetence of the armadillo to *M. leprae* (D. T. P., G. P. W., E. E. Storrs and C. Gannon, unpublished).

The lepromatous leprosy that develops in the armadillo involves internal visceral organs and tissues that are near the body surface¹. The cool body temperature of 30° C to 35° C of the armadillo enables lesions to develop in viscera, whereas in humans lepromatous lesions are generally limited to superficial cool regions.

Cellular immunity may be modified by changes in temperature, for instance, an increase in body temperature could possibly enhance cellular immunity. The role of fever as a potentiator of cellular immunity against malignant neoplasms and infectious agents needs to be assessed. Furthermore, the impact of cool temperatures also needs to be assessed as related to susceptibility to other infectious agents.

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Die-back of *Pistia stratiotes* on Volta Lake, Ghana

ANNUAL die-back of *Pistia stratiotes* L., associated with an epidemic of an aphid-transmitted virus infection whose symptoms are leaf yellowing and brittleness, was recently reported for the Ibadan area of Nigeria¹. It was supposed that similar virus epidemics may have checked the initial outburst of the weed on the Volta Lake in Ghana. We confirm that *Pistia* has died back on the Volta Lake from a maximum cover of about 4% of the lake surface in 1966, 2 yr after the dam was closed, to about 1% now (1973). In recent work on the Pawmpawm arm of the lake we have measured the rate of retreat of *Pistia* mat from the main lake. By marking flooded trees near the edges of the mat, it was possible to survey the limits of *Pistia* in August 1971, April 1972 and in July 1973. These surveys, together with an aerial photograph taken in October 1968 (P. C. Pierce, personal communication) made possible the construction of the map shown in Fig. 1, from which it can be calculated that *Pistia* receded at an average rate of about 7 ha yr⁻¹ over the period of observation.

In 2 yr of regular observations², *Pistia* retreat resulted from disruption of *Pistia* mats by storms in the late dry season (February and March), following several months during which the plants, even in enclosed permanent plots, had been severely weakened by poor growth. Aphids were rare in the populations observed, and there was no evidence that virus disease was of primary importance in causing leaf yellowness or brittleness. Size and rapidity of fluctuation in lake-level and antagonism by other plants, which have been implicated in *Pistia* die-back in other areas^{3,4,5}, also seem to be unimportant on the Volta Lake. Retention time here is 4 yr as against 76 d for Lake Kainji, Nigeria³; in any case, the stranding of plants in the drawdown area

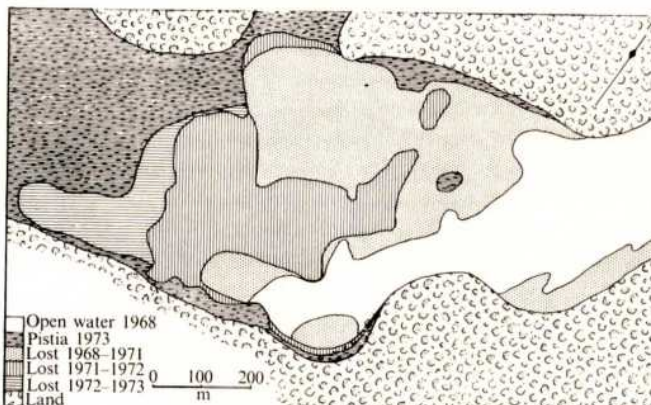


FIG. 1 Die-back of *Pistia stratiotes* on the Volta lake, Ghana.

should favour seed germination⁶ and is therefore unlikely to be responsible for disappearance of *Pistia*. The plant has not been replaced for the most part by any antagonising weed.

Circumstantial evidence suggests that poor growth of *Pistia* during the dry season may result from low nutrient status of the lake-water at this time, because of lack of rain to wash in nutrients from adjoining land. Lake nutrient status is probably improved during the early rains as a result of enhanced mineralisation by re-wetting of previously dry soil⁷, inflow to the lake of nutrients from such mineralisation and, possibly, direct enrichment of the lake by nutrients carried in early rainwater⁷. *Pistia* is known to respond sensitively to substrate nutrient level⁸, and especially to nitrogen⁴ the level of which increases many-fold as a result of re-wetting of dry tropical soils. Explosive growth of *Pistia* on the Volta Lake occurs following the first rains after the main dry season². There is evidence⁹ to show that nutrients such as phosphates have now dropped to less than half the level they reached when the lake was first formed.

The long-term disappearance of *Pistia* from the Volta Lake may be reflecting a change in the nutrient level, and probably also the decay and disintegration of flooded trees which originally helped to anchor *Pistia* mats.

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Progress of a breeding project for non-human primates in Colombia

THE International Primatological Society¹ and several recent publications²⁻⁸ have pointed out the urgent need for conservation of non-human primates. The capture of live primates for exportation is a major drain on natural populations and breeding programmes are a vital need which has been emphasised by two recent events. First, the Institute for Development of Renewable Natural Resources in Colombia has established limits and standards for the hunting of non-human primates⁹. Second, a large scale, international research project has been launched through a grant from the US National Institutes of Health to the Institute of Laboratory Animal Resources of the US National Academy of Sciences, which will tackle critical questions of supply and demand, and seek to identify primates which must be bred to meet research requirements¹⁰. (In 1969, 16,295 New World primates and 42,514 Old World primates were sold in the United States solely for research³.) We report here preliminary results of the first attempt at large scale, controlled breeding of New World primates in the natural habitat of an exporting country, and discuss the feasibility of future projects.

In 1967 Mike Tsalickis, an animal dealer in Leticia, Amazonas, Colombia, initiated a breeding experiment with squirrel monkeys (*Saimiri sciureus*), the New World primate most widely used in research. (*Saimiri* constitutes 10–80% of primates exported to the United States from Latin America³.) Between 1967 and 1970, Tsalickis released 5,690 monkeys (1,200 males and 4,490 females, some judged to be pregnant) on Isla de Santa Sofia II which was previously uninhabited by non-human primates. (Three adjacent fluvial islands under Colombian jurisdiction called Santa Sofia are designated Santa Sofia I, II and III.) The monkeys could not leave the island without swimming at least 1 km, and hunting and trapping were prohibited. In 1971 Tsalickis, on the basis of a sample count of females with young, estimated the birth rate at 80%. Assuming no mortality, he calculated the existing population at 20,698^{11,12}.

Santa Sofia II is a 1,000 acre (400 hectare) island in the middle of the Amazon River 33 km upriver from Leticia, formed by fluvial deposition perhaps as recently as 40–50 yr ago. Lakes devoid of trees form one-third of the island which is uninhabited by monkeys. (The level of the river fluctuates nearly 7 m seasonally, more than 90% of the island may be inundated during the peak of the wet season in April and May.) Another third is formed by low areas and the edges of the island which are covered by a successional community including *Cecropia* sp. The remaining third of the island is formed by the central and higher areas which are covered by a more advanced successional stage with a mixed community of non-deciduous trees. About 10% of the island has been cleared for agricultural purposes, including fruit trees (banana and guava) planted for the breeding project. Several areas were sporadically provisioned with fresh fruits and monkey chow to supplement natural food available.

From June to early August of 1972 we conducted a census of the *Saimiri* population on the island. Our primary objectives were: (1) to measure the population as accurately as possible; (2) to make recommendations concerning collection and (or) other aspects of population management; and (3) to demonstrate that multidisciplinary efforts can enhance the value of field studies, and that for projects of this sort they may be essential. In this report we describe briefly our census methods and results and their implications. (We will publish a more detailed report later¹³. In addition a report of a subsequent project conducted independently by R. C. B. on the monkeys of Santa Sofia II is in preparation and will provide further data pertinent to the present discussion.)

Our census procedure was chosen to accommodate the topography, vegetation and seasonal flooding; the behaviour and ecology of *Saimiri*, and the availability of time and personnel. To measure the total population we needed to estimate (1) the number of groups on the island, and (2) average group size. We first divided the island into three workable sections (areas A, B and C), aided by the presence of natural ecological barriers such as permanent large ponds (Fig. 1). We then cut sixty-one trails, forty-five at 100 m intervals and sixteen at 200 m intervals, the distance between trails depending on type of vegetation and resultant local visibility. All our trails were perpendicular to and connected by three trails cut by Tsalickis, and facilitated access to most of the island land mass (Fig. 1). The number of groups of *Saimiri* was estimated by repetitions of a synchronic multi-strip census, whereby four to seven field workers walked blocks of adjacent trails, locating groups, until all the trails in an area had been covered in one continuous period. Data on group size, direction and rate of group movement, group cohesion and intergroup distance were also gathered by each observer to aid in determining average group size, and to avoid counting groups more than once. The average group size was estimated in two ways: (1) by averaging accurate

TABLE 1 Population estimates based on two approaches to group size

Area	Average group size	Average No. of groups	Extrapolation of population estimate
A	41	10	410 (in 42% of land mass)
B			287 (in 30% of land mass)
C			269 (in 28% of land mass)
Total			966

Area	Estimated group size	Average No. of groups	Population estimate
A	50	10	500
B	50	4	200
C	50	3	150
Total		17	850

counts of several groups in area A, the most accessible area; and (2) by using field observation from all three areas. The total population of the island was also estimated in two ways. (a) The population of area A was determined by multiplying its average group size (method (1)) by the total number of groups in that area. Assuming comparable density throughout the island (on the basis of similarity of habitat), we then extrapolated to areas B and C on the basis of land area. The three area populations were then added to obtain the total island population. (b) The total island population was obtained by multiplying the estimated average group size (method (2)) by the total number of groups in all three areas.

Table 1 shows the total island population estimates obtained by the two methods. Method (1) yielded a population of 410 for area A, and a total island population of 966. Method (2) yielded a total island population of 850. Areas A and B were each censused seven times under varying conditions (time of day, weather, composition of observing teams and direction of movement of observers). Area C was censused twice. Approximately 350 man hours were spent in direct censusing. The variability in number of groups sighted was low (s.d. = 1.13), indicating relative consistency and lending confidence to the method. (Continuing field research by R. C. B. on Santa Sofia II generally supports the number of resident groups estimated during the summer of 1972.) We therefore conclude that the *Saimiri* population of Isla de Santa Sofia II as of August 1972 was between 850 and 966. Based on variance in our counts (s.d. = 1.13), the probability that there were more than 1,200 or fewer than 550 monkey on the island is less than 0.0001.

Our results have two possible interpretations: (1) if published estimates are reliable¹¹ there was a decline from 20,698 to 850-966 in 18 months, or (2) previous estimates are unreliable. We are not aware of any unusual or altered conditions such as flooding or disease which might account for such a drastic mortality during the 18 month interval, nor

has Tsalickis indicated such conditions. Previous estimates of population size were based on too little evidence and even then were too optimistic because the calculations assumed no mortality. In either of the interpretations census figures must be viewed in relation to the documented number of animals introduced to the islands (5,690). Substantial decline is evident.

We can only speculate as to causes of mortality, particularly since there is no way of knowing when and over what period the decline occurred. Nevertheless, clearly many important aspects of *Saimiri* behaviour and ecology were not known or not taken into account when this breeding experiment was planned. Factors that need to be considered include abundance of food, spatial requirements, predator pressure, disease, climatic change, consequences of introducing alien groups of unfamiliar and unrelated individuals, and the effects of a continued influx of new animals on established groups.

M. Tsalickis has communicated his personal conviction that large numbers of monkeys were stolen from the island for sale in Leticia. The only evidence of poaching of which we are aware was reported to us by T. Jerkins of Tarpon Zoo, Tarpon Springs, Florida. In October 1972 an unspecified number of monkey traps were discovered on the island by Tsalickis' employees. On February 26, 1973 a tattooed monkey from the island was discovered in a shipment to the zoo. This is evidently one of the animals introduced to the island when only a small portion were tattooed. (No tattooed monkeys were found in the 1972 census and the subsequent study by R. C. B.) On the basis of present information, we consider the poaching hypothesis both unverified and unlikely, although it cannot be excluded as a possibility.

The single most important lesson to be learned from the Santa Sofia experiment is that controlled breeding of non-human primates on a large scale cannot be accomplished without considerable basic biological data to support careful planning and management—even when such projects are conducted in apparently representative habitat within the natural range of a species. The successful breeding of *Saimiri* in the enriched tropical hammock of the Monkey Jungle, Miami, Florida, has indicated that controlled breeding of certain non-human primates in a natural or seminatural environment is possible, when careful management, including daily dietary supplementation, is provided. Although they live in only a 4 acre area, the squirrel monkeys of Monkey Jungle increased from thirty-seven animals to approximately 150 in 6 yr. The original thirty-seven were handpicked and in apparent good health. The population was fed seven times a day, 7 d a week, to supplement the natural diet¹⁴. In this connection it is important to note that Monkey Jungle is a commercial zoo and not a primate ranch. For that reason, and in spite of its success, it cannot answer unequivocally the question of whether or not *Saimiri*, or any other species, can be bred economically, that is, at prices competitive with current sources of non-human primates.

The *Saimiri* breeding project of M. Tsalickis on Santa Sofia II is provisionally licensed as a Zoo-Criadero (faunal breeding farm) by the Division of National Parks of INDERENA. Tsalickis' effort has paved the way for future primate ranching attempts, and continued studies on Santa Sofia will help determine more of the critical factors in *Saimiri* husbandry. Finally, the Santa Sofia experiment has again emphasised the practical need for more data on the behaviour and ecology of non-human primates. Such data are essential for the economical, large scale husbandry which must be achieved if many species are to avoid depletion in nature and are to be preserved as a renewable natural resource.

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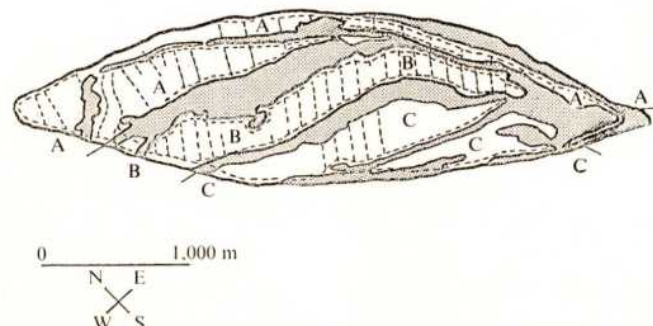


FIG. 1 Map of Isla de Santa Sofia II showing major survey areas (A, B, C), trails (dotted lines), and open water or marshy areas generally not inhabited by *Saimiri* (stippled).

and local housing and transportation. Financial support was also provided by the National Institutes of Health (to L.E.S.), Cornell University (to L.E.S. and D.S.B.), the US National Science Foundation (to R.A.M.), the Hooton Fund (to R.A.M.), and the Hunsaker Foundation (to R.C.B.). We also thank J. I. Hernandez, R. W. Cooper, and D. Hunsaker II of INDERENA for their help.

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Tobacco smoking and a 'stimulus barrier'

ONLY one in four smokers who wish to stop, actually succeed¹. The strength of the habit is hard to explain in terms of learning theory, since its manner of reinforcement is uncertain. This study considers a possible mechanism through which smoking might be positively reinforced².

Freud³ described a perceptual function which enables us to select from an overwhelming mass of sensory data only those aspects of the environment which have meaning or

significance. In this way, a large sector of potential sensory input is 'blocked out'. He therefore conceived this function as a stimulus barrier; 'a special integument or membrane which keeps out the stimuli'. Relative failure of this 'barrier' is unpleasant, and part of common experience. Irrelevant sounds intrude into consciousness, and are irritating.

This 'barrier' can be conceived in terms of neurophysiological models for the habituation of the orienting response⁴. The orienting response consists of a number of behavioural and physiological changes induced in the subject by a novel stimulus. This response diminishes and ultimately ceases after a number of repetitions of the stimulus. The individual is then said to have habituated to that particular stimulus. A new stimulus re-evokes the orienting response, causing dishabituation. It is not unreasonable to suppose that the phenomenon of habituation reflects an individual's ability to 'block out', or to selectively not attend to irrelevant stimuli. The possibility that smoking may increase the effectiveness of a 'stimulus barrier' was examined by a study of the habituation of the orienting response in smokers.

The subjects were 10 young men (20–25 yr of age) who habitually smoked at least one packet of cigarettes a day. Alpha-desynchronisation of the electroencephalograph was the aspect of the orienting response chosen for study, since the peripheral effects of nicotine may have altered the galvanic skin response, which is more usually chosen.

The EEG was recorded in a sound-damped room. An active silver-silver chloride electrode was set at the vertex (cz on the international 10/20 system) and referenced to the left mastoid. The subjects were tested on four occasions at three week intervals. The first session involved familiarisation with the recording procedure. Two series of stimuli were presented through loud-speakers 2 feet from the subjects. The first series consisted of twenty loud (90 dB) brief (0.5 s) bursts of pure tone (2000 Hz) with interstimulus intervals varying between 30–80 s. The second series differed only in that the frequency of the tones was 1000 Hz. Each series ended with a dishabituating tone of 10 s duration. The habituation point was defined as the number of stimuli at which the EEG showed no desynchronisation to a stimulus, when there was also no desynchronisation to the two succeeding stimuli.

The percentage of alpha activity was determined between the fifteenth and sixteenth stimuli, and done after the recording session by one observer who was unaware of the smoking conditions. Skin resistances were recorded as described elsewhere⁵, and the heart rates taken by means of cardiometer coupler (9857) on an Offner-Beckmann dynograph.

Before the second session, subjects were instructed to smoke according to their usual pattern. The 2000 Hz series of tones was presented while the EEG was recorded. Forty-five minutes later, subjects were instructed to smoke two cigarettes of their preferred brand and were then presented with the 1000 Hz series of tones. The mean duration between the last cigarette smoked before the session and the onset of recording was 31.1 min, s.e. 3.1, with a range of 20–50 min.

Before the third session, subjects were instructed to abstain from cigarettes for 12 h. The two series of tones were again presented, one before smoking, and the second after smoking two cigarettes. The fourth session was identical to the third except for the substitution of a nicotine-free placebo cigarette (roasted chicory leaves in authentic cigarette packing) instead of the preferred brand. The subjects were told the cigarettes were 'foreign', and none of them realised that they were not smoking tobacco.

The results (see Table 1) indicated that the immediate effect of tobacco smoking was to greatly increase the speed of habituation, and that placebo smoking had no effect.

Thus, in the second session, the mean habituation point changed from 16.1–4.5, after smoking (paired $t = 7.74$,

TABLE 1 Means and standard errors for habituation point, heart rate, skin conductance and proportion of alpha

		Session 1 (Training, series 1)	Session 2		Session 3		Session 4	
		Non-deprived	Non-deprived	Non-deprived + cigarette	Deprived	Deprived + cigarette	Deprived	Deprived + placebo
Habituation point	Mean	16.9	16.1	4.5	13.8	5.6	14.7	13.7
	s.e.	1.5	1.6	1.0	1.5	1.3	1.7	1.8
	<i>n</i> *	8	10	10	10	10	10	10
Heart rate (beats/min)	Mean	74.4	70.4	72.0	61.6	72.9	59.5	57.8
	s.e.	4.3	3.7	3.3	4.0	4.3	3.6	4.6
	<i>n</i>	9	9	8	10	10	8	8
Skin conductance $\mu\text{mho} \times 10$	Mean	85.9	104.4	136.3	75.4	122.7	103.4	106.6
	s.e.	23.8	33.9	27.8	22.6	79.2	22.8	36.6
	<i>n</i>	8	10	10	10	10	10	10
% Alpha	Mean	21.6	23.2	30.0	28.4	34.5	26.5	27.9
	s.e.	6.6	4.4	3.8	9.1	7.1	6.8	5.0
	<i>n</i>	8	10	10	10	10	10	10

* Although all 10 subjects participated in all tests, some readings had to be discarded due to recording problems.

$P < 0.001$) and in the third session, when the subject smoked after 12 h deprivation, the mean habituation point changed from 13.8–5.6 (paired $t = 4.62$, $P < 0.005$). On the other hand, during the placebo smoking session the mean habituation point remained virtually unchanged 14.7–13.7 (paired $t = 0.92$).

Delayed consequences of smoking were not manifest in habituation rate since there was no difference between the rates after a period of normal smoking and after a 12 h deprivation. The mean habituation point before smoking in the second session was 16.1, while the mean habituation point after deprivation was 14.3 (paired $t = 1.14$).

Occasional failures to show a dishabituating response were evenly distributed between tobacco-smoking and placebo-smoking sessions.

Since the change in habituation was not found with placebo, it seems likely that the effect was mediated by a drug, presumably nicotine, rather than by psychological factors. Increase in speed of habituation usually reflects diminished arousal, and drugs which effect such a change are therefore considered to be sedative⁶. Nevertheless, the immediate effects of smoking on habituation cannot be adequately explained in terms of sedation, since this is not reflected in measures of arousal.

Changes in peripheral measures of arousal suggested that smoking may cause mild central nervous system excitation rather than sedation. The skin conductance tended to rise, while a significant increase was shown in heart rate during session 3 (paired $t = 5.52$, $P < 0.001$). This increase was not apparent in session 2 (paired $t = 0.90$). There was also a significant difference between the heart-rates of deprived and non-deprived subjects before smoking (paired $t = 4.87$, $P < 0.001$). These changes, however, may be purely autonomic and not be correlated with cerebral events. Nevertheless, they are consistent with certain previous electroencephalographic evidence^{7–10}, although in the present study no significant change in proportion of alpha activity was induced by smoking (paired t in session 2 = 1.27, and in session 3, $t = 0.69$).

Theoretical models of the habituation phenomenon suggest that it depends upon active inhibitory mechanisms⁴, of which a number have been described^{11–15}. Clinical studies involving habituation and measures which are likely to reflect central nervous system excitation, suggest an inverse relationship between 'inhibitory' and 'excitatory' processes¹⁶. It therefore seems not impossible that tobacco smoking causes a dislocation of the usual relationship, so that inhibitory mechanisms are stimulated without simultaneous reduction in CNS excitation.

In conclusion, the findings suggest a possible means of positive reinforcement of tobacco smoking through its effect upon the individual's manner of screening of sensory input.

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Reaction of methyl mercury with plasmalogens suggests a mechanism for neurotoxicity of metal-alkyls

THE biosynthesis of methyl mercury from mercuric ion, by microorganisms which live in sediments polluted with mercury, is of special significance to the bio-accumulation of mercury^{1–3}. Although the mechanism of methyl mercury biosynthesis is understood to a large extent at the molecular level^{4–6}, very little is known about the mechanisms of molecular interaction in both the bio-accumulation and the neurotoxicity of this compound. It is clear that ingestion by humans of food contaminated by methyl mercury leads to

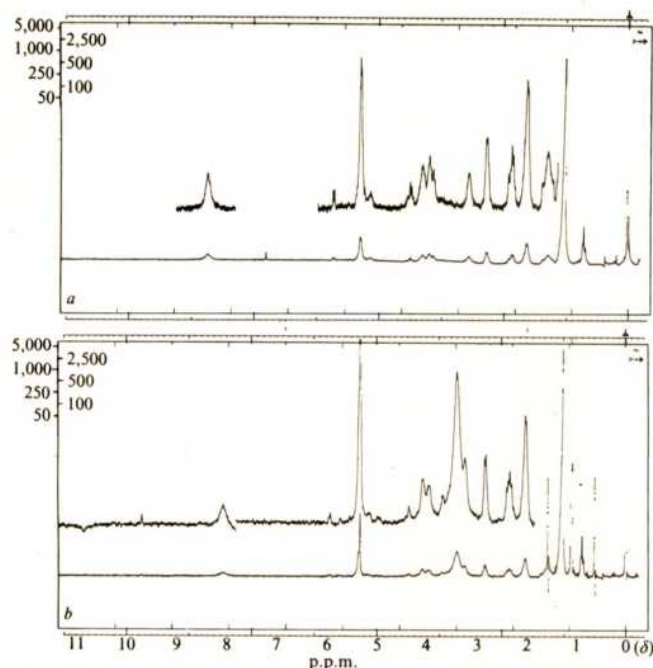


FIG. 1 *a*, 220 MHz NMR spectrum of plasmalogen. *b*, 220 MHz NMR spectrum of the products formed when plasmalogen is incubated with methyl mercury chloride and water.

the synthesis of methyl mercury chloride in the stomach, and this, being very non-polar, is easily transported into the blood stream⁷. The distribution of methyl mercury in animals is characteristically different from that of inorganic mercury, in that alkyl mercury compounds have a tendency to partition into the lipids, or hydrophobic regions of the cell⁸.

Post mortem examinations of victims of the Minamata disaster, who suffered neurological disease after eating fish contaminated with mercury, revealed extensive damage to the central nervous system. Widespread lysis of cell membranes, especially of neuroglia and granule cells, was reported^{9,10}. Methyl mercury in aqueous systems is an unreactive molecule, and it is hard to see how it could cause such irreversible damage. Although methyl mercury reacts readily with sulphhydryl groups, it does not form stable complexes with nitrogen-containing bases in aqueous solution; in fact it does not complex with pyridine in aqueous solution¹¹.

It is possible that the reaction of methyl mercury with cysteine residues, which are present at the active sites of several important enzymes of the glycolytic pathway, could provide a basis for the neurotoxicity of this compound¹². Concentrations of methyl mercury greater than catalytic concentrations would have to be present, however, to be effective in enzyme inhibition. As the binding of methyl mercury to thiols is reversible, relatively large concentrations of methyl mercury would be required for that enzyme inhibition. Furthermore, inhibition of the enzymes in the glycolytic pathway would have an indirect and reversible effect on membrane synthesis. We report here that methyl mercury can react both catalytically and directly with a

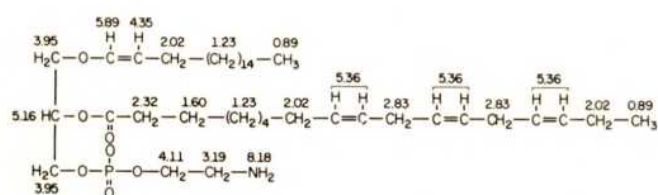


FIG. 2 Structure of plasmalogen with 220 MHz NMR proton assignments in δ (p.p.m.) adjacent to each carbon atom.

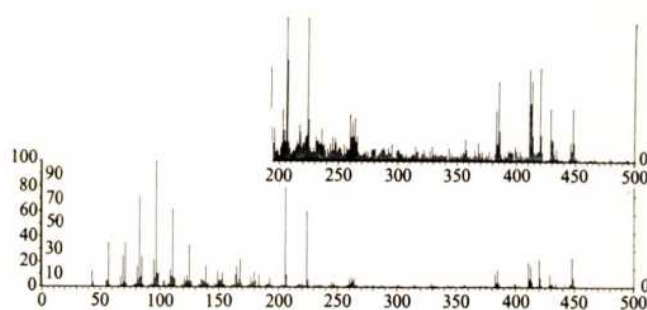


FIG. 3 High resolution mass spectra of the 2,4-dinitrophenylhydrazine derivatives isolated from the reaction products of the methyl mercury-catalysed hydrolysis of plasmalogen. Spectra were recorded at 10 eV and at 70 eV (inset).

group of phospholipids which are important in membrane structure for cells of the central nervous system.

High resolution nuclear magnetic resonance (NMR) has been used to assign all the protons of a plasmalogen (L α phosphatidyl ethanolamine), which has palmitic and stearic acid as alternative residues to the vinyl ether linkage at the α' carbon, and linolenic as the unsaturated fatty acid at the β carbon. Methyl mercury chloride is soluble in this phospholipid, and the methyl mercuric ion catalyses rapid hydration and hydrolysis of the vinyl ether linkage to give a mixture of palmitic and stearic aldehydes plus the linolenic monoglyceride product. The course of this reaction was followed by 220 MHz NMR, and the aldehyde products were characterized further by mass spectrometry of their 2,4-dinitrophenylhydrazine derivatives. The relevance of this reaction to the neurotoxicity of metal-alkyls is probably linked with membrane lysis, which is specific for those membranes which contain plasmalogens as a major constituent of the phospholipid backbone in membrane structure.

Reaction mixtures were set up containing 24.1 mg of plasmalogen (phosphatidyl ethanolamine obtained from P-L Biochemicals) dissolved in 1.0 ml of CD_2Cl_2 to which 163.5 mg of methyl mercury chloride was added. When 0.17 ml of water was added the reaction proceeds at 25°C to yield palmitic and stearic aldehydes. We used a concentration of methyl mercury chloride twenty times greater than that of plasmalogen, but the catalytic nature of the reaction makes it possible to use much less. Decreasing the concentration decreases the rate of catalysis without altering the stoichiometry for the reaction.

Fig. 1 shows the 220 MHz NMR spectra of the plasmalogen before and after addition of methyl mercury chloride. From the spectrum in Fig. 1*a* it is possible to assign all the protons in this mixture of compounds. Fig. 2 shows the basic structure of the plasmalogen with resonances assigned in δ (p.p.m.); using tetramethylsilyl as an internal standard. The spectrum in Fig. 1*a* was integrated and the plasmalogen structure confirmed by these NMR data. Resonances of special interest in the overall reaction with methyl mercury chloride are those due to the protons of the *cis* double bond of the vinyl ether linkage which are assigned at $\delta = 4.35$ and $\delta = 5.89$ respectively (Figs 1*a* and 2). Also the resonance at $\delta = 8.18$ which represents the protons of the primary amine of ethanolamine is interesting because this resonance shifts from $\delta = 8.18$ to 8.07. This shift is indicative of a weak interaction between methyl mercury and this basic amino group; an interaction which does not occur in aqueous solution. Therefore, the hydrophobic nature of the phospholipid facilitates coordination to nitrogenous bases to some extent. When methyl mercury chloride is added to the plasmalogen, the vinyl ether resonances at $\delta = 4.35$ and $\delta = 5.89$ decrease in intensity and a new resonance which is characteristic of the presence of a long chain aliphatic aldehyde appears at $\delta = 9.69$ (Fig. 1*b*).

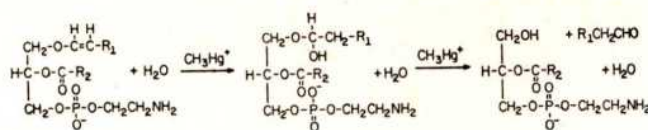


Fig. 4 Proposed reaction sequence for methyl mercury-catalysed hydrolysis of plasmalogen.

The aldehyde products formed in this reaction were characterised as their 2,4-dinitrophenylhydrazone derivatives. The reaction products from the NMR experiment were transferred to a reaction vessel and allowed to react with 1.0 ml of 0.2% 2,4-dinitrophenylhydrazine in 2N H_2SO_4 . The reaction was allowed to proceed for 1.5 h and then the products were extracted into chloroform. The chloroform layer was dried over anhydrous sodium sulphate and evaporated to dryness. The yellow residue was dissolved in 0.5 ml of benzene and applied to a silica gel column (2 cm \times 17 cm). The column was eluted with benzene, and 5.0 ml fractions were collected. Two yellow compounds were eluted from the column, and the first fraction to elute was evaporated to dryness and subjected to mass spectrometry (Fig. 3). Molecular ions at m/e 420 and m/e 448 are consistent with the product being a mixture of palmitic and stearic aldehyde 2,4-dinitrophenylhydrazones. The fragments at m/e 385 and m/e 413 are due to the loss of H_2O and OH from the palmitic and stearic derivatives respectively. The peaks at m/e 224 and 206 are due to McLafferty rearrangements involving three carbons of each aldehyde chain, and the 2,4-dinitrophenylhydrazone portion of each derivative¹³. The rest of the fragmentation pattern is consistent with that of a mixture of C_{16} and C_{18} saturated aliphatic carbon chains.

High resolution NMR is especially valuable for the study of the structure and reactivity of phospholipids in solution because all the protons for these complex molecules can be assigned. We have shown that methyl mercury reacts catalytically in promoting hydration and hydrolytic cleavage of the vinyl ether linkage in plasmalogens to give a mixture of long chain saturated aldehydes. A sequence for this reaction is proposed in Fig. 4. The fact that methyl mercury is soluble in phospholipids, and causes lysis of certain cell membranes in the central nervous system makes it tempting to speculate that this reaction could have significance in the neurotoxicity of this metal-alkyl. Also, it is expected that other metal-alkyls should catalyse this reaction by a similar reaction sequence.

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GENERAL

Hydrogen fuel

HYDROGEN is receiving much attention as a clean fuel¹⁻⁴. It is usually stated or implied that hydrogen is a clean fuel because only water is produced when hydrogen is ignited in an engine. Unfortunately, this is not a fact. The following reaction also occurs:



Furthermore, as energy is extracted from the reaction as work, the reaction favours the production of more hydrogen peroxide.

A one-cylinder Briggs and Stratton engine was equipped with a propane-type carburettor, and the muffler was replaced with a stainless steel water-cooled condenser 3 feet in length. A dual feed system enabled it to be started using propane. When the engine had reached 1,750 r.p.m. and was running smoothly, the fuel was slowly changed from propane to hydrogen while the engine speed was maintained. After the engine had been running on pure hydrogen for 10 min the condensate from the exhaust was collected and analysed for H_2O_2 .

Qualitative tests with $\text{TiOSO}_4\text{-H}_2\text{SO}_4$ solutions and with Tes Tape urine sugar analysis paper indicated a strong positive for H_2O_2 . The concentration was analysed using a standard KMnO_4 titration. The results of three separate runs are given in Table 1.

TABLE 1 Concentration of H_2O_2 in the exhaust of an engine using H_2 gas as a fuel

Test No.	H_2O_2 in the exhaust (p.p.m.)
1	230
2	220
3	220

Although the concentration of H_2O_2 in the exhaust was not high in these experiments, the implications of using a large number of big engines producing H_2O_2 in the environment must be considered. Hydrogen peroxide is known to generate free radicals which would be a potential hazard if inhaled. If they are to be completely safe, engines operating on hydrogen gas will probably require some type of catalytic converter for the exhaust gases.

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book reviews

Men from beyond Mars

Communication with Extraterrestrial Intelligence. Edited by Carl Sagan. Pp. xx+428. (MIT: Cambridge, Mass. and London, November 1973.) \$10.

IN sight of Mount Ararat, reputed resting place of Noah's Ark, scientists from six countries met to confer on "Communication with Extraterrestrial Intelligence" (CETI) in September 1971. The 54 delegates concluded: "It seems to us appropriate that the search for extraterrestrial intelligence should be made by representatives of the whole of mankind".

This volume is a transcript of the conference, which was held at the Byurakan Astrophysical Observatory in Soviet Armenia. The observatory's director, Viktor Ambartsumian, was chairman of the Soviet delegation.

The first-ever CETI conference was an unofficial gathering at Green Bank, USA, in 1961, at which Frank Drake presented the now classic formula for calculating the probability of communicative life in the Galaxy, which has since been discussed and elaborated in various papers and books. More than half the Byurakan proceedings consist of a wide reassessment of the many implications in the formation of planets, origin of life, rise of intelligent life and the desire to communicate. Contributors to the debate include Francis Crick, Tom Gold, V. I. Moroz, Leslie Orgel and Y. N. Paryisky.

Surprisingly, the Byurakan estimate differs little from previous suggestions. "We have estimated the rate of formation of such civilisations in the Galaxy: one is formed about every 10 years", says Carl Sagan, the United States delegation's head, in summary of the debate.

Yet the problem of estimating the lifetime of such a civilisation remains the greatest unknown in any such calculation: "If civilizations destroy themselves shortly after arising, there may be no one for us to talk to but ourselves", says Sagan. Canadian anthropologist Richard Lee argues for the positive effect of CETI on a civilisation: "If we receive a communication we want to stick around to see what the reply is".

"There are a million technical civilisations in the Galaxy. This corresponds roughly to one out of every hundred thousand stars", Sagan concludes cautiously on page 166, freely admitting the possible massive error in this figure. A

few pages on, Sebastian von Hoerner makes his own estimate—600 light years—of the distance between current communicative civilisations.

Startling news to come later in the conference was V. S. Troitsky's revelation (page 256) that the Andromeda galaxy and 11 stars near to Earth, including Tau Ceti and Epsilon Eridani, had been monitored in autumn 1968 for artificial signals. This was the first attempt since Drake's project Ozma to eavesdrop on stars. A proof note is added to outline the recent similar work of G. Vershuur (*Icarus*, 329; 1973) and another United States project. All such projects have had negative results.

Recently, N. S. Kardashev and Troitsky have reported possible artificial signals from space, breaking Iosef Shklovsky's rule of thumb presented at Byurakan: "We must follow the legal principle of presuming signals natural until proved otherwise".

Several papers have already been spun off from discussions and presentations at the CETI conference, including one by Sagan (*Icarus*, 350; 1973) which is reprinted as an appendix. Also previously published are Freeman Dyson's free-ranging presentation to Birkbeck College in 1972, *The World, The Flesh and The Devil*, and the CETI conference resolutions (*Icarus*, 412; 1972).

Another topic discussed at Byurakan was astroengineering—large-scale projects of advanced civilisations that might produce tell-tale signs such as extensive amounts of infrared emission. Techniques of contact with extraterrestrials were outlined, with Bernard Oliver summarising the project Cyclops report (NASA CR 114445). Should such an expensive undertaking be funded? Oliver is in no doubt: "All past human history may indeed be merely a prelude to an inconceivably exciting future as participants in a galactic culture".

Linguist B. V. Sukhotin reveals the development of a decoding programme, in a short section on the possible contents of extraterrestrial messages. Then follows a brief discussion involving historian William H. McNeill on the consequences of such contact. "We need from the beginning to accept responsibility for the effect of what we may be planning", says Philip Morrison. Sagan and Morrison have since taken part in another discussion on ETI with participants including a theologian, a philosophical viewpoint not represented at Byurakan.

The conference resolutions outline a number of directions for future research, and suggest new radio and infrared instruments to aid the search. (The Soviet 600 m radio telescope to be opened soon will be partly used for CETI research.) "All of the instruments described above have the capability of providing important data in subjects quite separate from CETI", the participants point out.

But it was Shklovskii who summed up their main problem: "All the natural sciences rely upon observations and experiments. We have none of that here".

IAN RIDPATH

School to college

Continuity and Discontinuity: Higher Education and the Schools. Pp. xi+116. (A Report and Recommendations by the Carnegie Commission on Higher Education, August 1973.) (McGraw-Hill: New York and London, October 1973.) £2.95.

REPORTS on education are not, as a rule, noted for their attractive reading. This one is an exception, partly on account of its stimulating and imaginative mode of presentation, and partly because of the fascinating light it throws on some of the more intransigent educational problems that face us in Britain today. It is sometimes comforting to see one's own difficulties painted larger than life in the colours of another country. Chapters 2 and 3 of the report provide a condensed history of American educational evolution dating from 1850 and projected into the year 2000. Some outcomes are an emphasis on the need for more closely coordinated admissions policies from school to college, the need to experiment with D. Arts degrees as teaching alternatives to the PhD, and the necessity for revised and more standardised testing procedures. Chapters follow on the reform of the admissions procedure to college, improvements in secondary education, new structural patterns relating to such factors as course content, the education of school teachers and administrators, and possible mechanisms for better school-college collaboration. Chapter 1, being a statement of major themes and recommendations, seems somewhat out of place and would have been better at the end as an outcome of all that had gone before.

Several sections of the report are of particular interest at the present time:

for instance, a scheme for shortening the length of the college course to produce a BA qualification somewhat similar to our proposed Dip.HE. It is interesting to be told that teacher education is now regarded as the largest single enterprise in American higher education. Clearly, many of the problems in this field, with the teacher training curriculum, teaching practice arrangements in schools, and in-service training closely parallel our own.

The report will thus be of interest to anyone concerned with the planning of secondary or higher education. Reading it, I could not help but feel profoundly thankful that Britain is a relatively small nation with a public examination system and entry requirements to higher education subject to some degree of central control and hence an equivalence of standards.

W. H. DOWDESWELL

Glimmer of hope

Priorities for Action: Final Report of the Carnegie Commission on Higher Education, with Technical Notes and Appendixes. Pp. x+243. (McGraw-Hill: New York and London, October 1973.) \$4.95.

THE ability of the British to look into their future by looking into the American present has been disturbed by the advent of instant communication, through which the American present influences not only their future but also their present. They therefore face the paradoxical situation that while in the sphere of mass higher education they have not yet caught up with the United States of the 1950s, they share in the disillusionment and graduate employment problems of the 1970s. "A traumatic loss of sense of assured progress has occurred (page 6) the world over, and as a result the British are in danger of accepting their inferiority to the educationally developed nations permanently and disastrously. The report, with its dispassionately balanced outlook, provides an effective antidote to this policy of despair.

"In 1870, the issue was the modernisation of higher education—how to adjust to industrial and agricultural expansion, to political populism, to the rise of science. To-day the issue is more the humanisation of higher education—how to respond to the greater aspirations of more individuals for a higher quality of life, how to adjust to the social facts of more affluence and more leisure, how to incorporate into higher education the rise of the creative arts." (page 21.)

"One of the interesting—and, to some, surprising—discoveries of recent times has been that quality and quantity are

not necessarily inconsistent with each other in the academic world". (page 27.)

"We have suggested special admission provisions for disadvantaged students where their ability and the special assistance of the College will make possible their meeting, in full, the academic standards of the college within a reasonable period of time and certainly by graduation. Remedial work will be necessary." (page 37.)

"Planning for the future of higher education should be on a contingent basis, subject to constant re-examination. . . . The technocratic planning analysts are bound to be wrong." (page 80.)

Finally and rightly, the report quotes Ashby's remark about the need to preserve the "thin clear stream of excellence" (page 30). But are we always sure that in pretending to protect excellence we are not protecting privilege?

The editor has restricted me to 400 words, and within that compass I cannot do justice to this remarkable report. The purpose of the disconnected quotations above is to persuade politicians and civil servants, industrialists and businessmen, academics and students, to read this remarkable report whole and to take heart from it.

L. R. B. ELTON

Pacific problems

Nature Conservation in the Pacific. Edited by A. B. Costin and R. G. Groves. Pp. xvi + 337. (Proceedings of Symposium on Nature Conservation in the Pacific of 12th Pacific Science Congress held in Canberra, August–September 1971.) (Australian National University Press: Canberra, April 1973.) \$12.50.

LIKE most other conference proceedings, this book has taken an unconscionable time to produce. Yet since Dr Lee Talbot says on page 261 that "relatively little progress on conservation problems has occurred in the South-east Asian region" since the Bangkok Conference of the International Union for the Conservation of Nature in 1965, perhaps this does not matter much. Nature conservation problems seem to be either static or dynamic in the wrong direction. For Dr Talbot goes on to add that most of the same problems that existed in 1965 are still problems, some of them considerably aggravated, while a whole range of new problems have appeared.

Although the subjects covered by the symposium are wide ranging, the eastern Pacific is almost wholly omitted, the northern Pacific is grossly underplayed, and Australia and its neighbouring region bulk as large as one would expect from the locale.

Many of these papers are excellent, but I wonder whether it is useful to the scientific community to have them all

printed, after an undue delay, in book form? Would it not be better to have an abstracting service for these conferences and symposia, widely circulated soon after the event, and enabling people interested in particular papers rapidly to get a copy?

RICHARD FITTER

Drug receptors

A Guide to Molecular Pharmacology-Toxicology. Edited by R. M. Featherstone. Part 1: pp. xiv+425; part 2: pp. xiii+426–811. (Modern Pharmacology: 4 series of Monographs and Textbooks, vol. 2.) (Dekker: New York, September and November 1973.) Part 1: \$29.50; part 2: \$18.75.

MOLECULAR biology, it is said, owes its origins in no small part to the application of novel physical and chemical techniques to biological problems. These books are intended to go one step further, by describing how several of the newer techniques of molecular biology are being used to solve interesting and important problems in pharmacology and toxicology. They contain a collection of 21 chapters by a total of 30 authors, each chapter devoted to model systems, methodology, or both. As the editor says, there is no great thread of continuity among the chapters and none was intended. Nevertheless, the books are aimed pretty squarely at proteins as drug receptors: witness the contorted sausage on the dust jacket and the categorical statement on page 308 that "whenever a drug receptor has been identified, it turned out to be a protein". (Workers on actinomycin beware!) This no doubt explains why there is no treatment of molecular interactions between drugs and nucleic acids, which to my biased view seems rather a pity.

In part I the first three chapters, on model membranes, sugar transport receptors and ultrastructural studies, provide a useful and balanced introduction to the next four which are largely concerned with receptor identification and isolation, the prospects for which are succinctly stated in the quotation from Professor Burgen at the head of chapter 4. Here is a mine of valuable information about progress in several important areas which will commend itself to many a graduate student embarking on research. There follow three chapters concerned with conformational changes induced in proteins by drugs, the molecular pharmacology of acetylcholinesterase, and enzyme kinetics in the service of studies on structure-function relationships.

The scope of Part 2 is broader, with more concentration on the application of physical methods and mathematical

approaches to pharmacological problems. It is noteworthy that several authors of the more theoretically-based chapters are at pains to put their contributions into historical perspective and to emphasise that their calculations are intended to complement, but in no way replace, experimental investigations. There are chapters on nuclear magnetic resonance, electron spin resonance, optical activity, molecular orbital theory, and thermodynamics as applied to the study of drug-protein interactions. Another three chapters deal with anaesthetics, including the noble gases, primarily from the standpoint of discussing intermolecular forces involving simple drug molecules. They are reinforced by another chapter outlining the use of genetic variants to help probe the sites of action of inert anaesthetic gases and the binding of oxygen to haemoglobin. The two remaining chapters concern the induction of specific proteins by steroid hormones and the relative usefulness of lipids and proteins as models for studies in molecular pharmacology.

Notwithstanding delays caused by the failure of one author to deliver the goods (and he is accorded most commendably charitable treatment by the editor in his preface) there is evidence that a real effort was made to publish these books as promptly as possible. The bibliographies are sufficiently full to give the reader ready access to much if not most of the primary literature, but the rudimentary nature of the subject index places severe limitations on the value of the work as a reference source or guide. There is a much larger author index, but this is of limited value except as a means of verifying that a particular author's work has been cited. The intention is, however, clear enough that this publication is conceived as a collection of self-contained essays and in that light it will serve a useful purpose. Its appearance in two parts is in keeping

with the idea that each part may stand alone, but since no index is provided in part 1, and pages in part 2 are numbered to follow consecutively those of part 1, the two books are not so much companion volumes as moieties of a single work. The price seems rather high, particularly for part 1, but no doubt remains within the budget of libraries and our more affluent colleagues.

MICHAEL WARING

Industrial research

Co-operative Research in Industry: An Economic Study. By P. S. Johnson. Pp. vii+232. (Robertson: London, April 1973.) £4.

THE cooperative research associations have been a relatively neglected sector of the British R and D system. From their inception they have had to struggle hard for their existence and 14 have failed to survive. Their financial basis has always been somewhat precarious, as governments have usually striven to reduce their own contribution and persuade industry to cough up more. Industry, on the other hand, has been generally reluctant to increase its share, partly because of competitive considerations which militate in favour of captive in-house R and D, and partly because of backward attitudes. Only during and shortly after the two world wars did a more generous and far-sighted approach prevail. The RAs indeed owed their very existence to the first world war, which demonstrated forcefully the extent to which some branches of British industrial technology had fallen behind German competition.

The RAs have also been relatively neglected by economists. Not since Edwards's book in 1950 has there been any serious scholarly discussion of the fundamental economic problems of co-

operative industrial research in a capitalist competitive economy. Johnson's modest but highly competent book is therefore extremely welcome. It is especially valuable because it sets the problem firmly in a historical context, permitting one to assess the oscillations in government policy in a long-term perspective. In addition to the two introductory historical chapters, the book includes a valuable statistical survey of RA expenditure in each branch of industry and of the detailed breakdown of RA activities, with many useful tables. The chapters on economic analysis are complemented by the results of a field survey on the views of company research directors, particularly in relation to the growth of contract research. Finally, there is a very brief chapter on RAs in Europe and the United States. This is by far the weakest chapter in the book, which is a pity, as international comparisons would be particularly valuable in view of the growth of EEC-based industrial research.

In his conclusions on methods of financing RAs, Johnson generally welcomes the recent trends which he has demonstrated in his analysis, particularly the growth of contract finance, the switch from general government grants to specific government grants, and the trend towards payment for particular services, such as technical information, testing and so forth. Though his analysis is well argued and generally sound, and also reflects the views of many RA members, my own view is that he may perhaps be missing the wood for the trees to some extent. His survey shows fairly conclusively, as others have also shown, that most RAs are providing some very useful infrastructural technical services, particularly technical information, abstracting and translation services, standards, testing and safety work. They also do some very useful background research which benefits these activities and the research programmes of member firms. In these circumstances, though the extension of contract research is certainly welcome, it does seem to me that cheese-pairing on the small amount of government support for the RAs can be carried too far.

My main criticism of what is otherwise an admirable book is that Johnson does not relate his study of the RAs sufficiently to the general context of government policy for British industrial technology. The failure on the part of some governments to recognise their inescapable responsibility for the health of British science and technology, the efforts to dodge this responsibility in 1970-1972, the lack of a consistent policy for government laboratories and other aspects of the industrial science-technology system are an essential

Wanted: prolific big name

Nature receives many more books than it is able to review, but book publishers accept this and do not seem to withhold books which will not necessarily get reviewed. In certain cases of very expensive books or very small publishing companies, the publisher will make an informal enquiry about whether the book will be likely to be reviewed, since the expense of sending a copy for it not to be reviewed may be less easily bearable.

Recently, however, we have received a press release from a very large publisher on two books on the history of astronomy due later this

year. The aim is a "luxurious quality" of production and, quaintly, they can only be offered as a limited edition because of, among other reasons, "the wealth of illustrative material in the text and the nature of the subject".

The two books are £15 for 400 pages and £30 for 800 pages—not an outrageous price for well illustrated books, but "you will appreciate that the number of review copies... is very restricted. It will help us to judge between conflicting claims if you can specify the reviewer's name and the space you are prepared to devote".

background to the somewhat miserly approach to the RAs. Consequently, Johnson never asks how it was possible for successive governments to squander over £1,000 million on Concorde, while they were making great efforts to save a few hundred thousand pounds on the RAs. But this is the question which must be asked if we are to get value for money from government expenditure on R and D, if British industry is to be able to compete in the 1980s, and above all if British science and technology are to contribute substantially to the improvement of the quality of life for most people in these islands.

C. FREEMAN

Superconductive magic

Superconductive Tunnelling and Applications. By. L. Solymar. Pp. xii+406. (Chapman and Hall: London, June 1972.) £8.00.

THE story has all the ingredients of scientific legend: The phenomenon of superconductivity was discovered in 1911, but nearly half a century of effort by many of the leading lights of theoretical and experimental physics was required before it was well and truly understood. A necessary but not sufficient condition was the invention of quantum mechanics; an almost immediate consequence of that invention was the uncovering and elucidation, as early as 1928, of the phenomenon of tunneling. Not until the early sixties, after the development of a successful microscopic theory of superconductivity by Bardeen, Cooper, and Schrieffer, were these two phenomena brought together. And, lo, there materialised a veritable cornucopia of scientific fruit, with implications and applications ranging from purest physics to manifestly relevant technology. Moreover, the principal architects of this fertile union, Ivar Giaever and Brian Josephson, were mere students, not yet ordained in the doctoral priesthood of science. We have recently seen their achievement crowned with the Nobel Prize, hard on the heels of the same laurel for Bardeen, Cooper and Schrieffer.

If this view of the birth of tunneling in superconductors and of the Josephson effects seems a bit romantic, it is because I, after a decade of rummaging among the contents of the cornucopia, still find the whole affair rather magical. The task which Dr Solymar has set himself in writing this book is to present the results of the first decade or so of research on superconductive tunneling, magic included, in a form accessible to final year undergraduates and postgraduates in physics and electronic engineering, and to specialists in other fields who wish to learn what all the fuss has been about and perhaps to contribute to

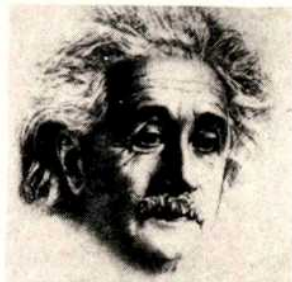
the continuing development of this field. This is no easy task. Much of the fundamental theoretical foundation is highly sophisticated. Although, after a decade of intensive work, it can fairly be argued that there remain few if any major mysteries in the field, contributions to knowledge of the basic phenomena continue to appear, and reports of advances in technologically relevant areas, especially of the Josephson effects, are proliferating beyond the ability of mortal man to keep track of, much less read and understand. Solymar's solution to the problem is to exploit a feature which superconductive tunneling shares with certain other physical systems, like the hydrogen atom: although one's study of the system can be carried to almost any level of complexity and sophistication, there exist levels at which real understanding, together with an appreciation for the beauty and the magic, can be conveyed to almost any student with a modicum of background.

He avoids the details of the microscopic theory, concentrates on phenomenological descriptions and models, and succeeds in presenting a comprehensive and understandable picture of the whole field in less than four hundred pages. It is all there: normal electron tunneling, Josephson tunneling, special effects, diagnostic and device applications, device fabrication, fluctuation phenomena, even a fascinating statistical note on the development of the field as measured by publications—number, journal distribution, country of national origin, and so on. It is a kind of Michelin green guide to the field. And for the reader who wants more than a two-paragraph or two-page introduction to the history of a particular château, there is a complete reference list (nearly 10^3 entries) to which he can go to learn everything that's known (or conjectured) on the subject.

As I understand it to be a reviewer's duty to carp, I am compelled to say that the relative emphasis on topics is sometimes not to my taste, that the urge to comprehensiveness has sometimes led the author to rather tedious listings of "X did Y experiment and measured Z", and that the proof reading leaves something to be desired (for example R. A. Ferrell's name consistently misspelled, the tin gap frequency reported at half its actual value (page 119), Figs 11.3 and 11.4 interchanged). On the whole, however, I think this book provides a remarkably good introduction to the field, one which I would (and will) recommend to any of my students or colleagues who wish to understand what superconductive tunneling is all about. I wish I had written it.

D. N. LANGENBERG

Einstein



ALBERT EINSTEIN
Philosopher-Scientist
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Paying the Civil Service Scientist

THE question of scientists' pay is not one for glib editorialising. Within the confines of a thousand words it is simply impossible to give a rational assessment of what should be done to the present simple pay structure for the enormously complex organisation that is the British Civil Service. What one can do is try and see broader issues. The contributions in *Nature* by Dr Leigh (248, 184; 1974) and Drs Gibson and Hawtin (in this issue) deserve careful reading because they clearly set out some of the fundamental questions underlying the present impasse.

In the late 1940s the Priestley Commission established that the pay of civil servants should be removed from the "cockpit of politics". To this end it proposed that parity be maintained with salaries outside the Civil Service and this, despite the inflexibilities of salary scales and increments, worked tolerably well for many years. Scientists were always in a potentially anomalous situation, since although they do not form a large percentage of all civil servants, they do account for more than half of Britain's scientific research manpower when scientists in fringe bodies (whose pay scales are coupled to civil service rates) are included. Far from avoiding pace-setting, which was Priestley's hope, scientific pay rates in the Civil Service cannot but set the pace for all scientists' pay. Up to 1971 the circularity inherent in this system was avoided by establishing broad parities between the scientific categories and their counterparts in the administrative, professional and technical grades. Since then the internal parities have been discarded and external parities sought through so-called 'pay research'. The result of this exercise did not satisfy the Institution of Professional Civil Servants, representing the scientists, and the matter is now with the Pay Board, whose decision is expected within weeks. There is no dispute, however, over the fact that scientists have slipped behind in internal parity during the dispute nor that they have conducted themselves with restraint. It is inconceivable that they should not be well rewarded by the Pay Board or that their forbearance should penalise them.

There are three salient points which need some emphasis: that going off an internal parity has created some nonsensical situations which deserve urgent attention; that the government, in deciding its own scientists' pay, is deciding the status of a profession; and that the role of government in science is worth continued questioning.

Every scientific civil servant can point to anomalies in structure. For instance scientists as they become more senior become more likely to administrate, and to main-

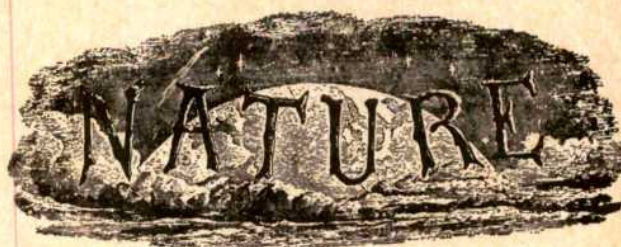
tain a distinction between administrators who have followed different paths to their present position is manifestly absurd. Nor is the anomaly confined to the upper echelons, for many scientists could easily have been classed as technologists and *vice versa*, yet there are now hundreds of pounds between comparable categories.

More important than repairing these frayed edges, though, it is vital that the question of civil servants' pay is seen for much more than that. Like it or not, the Pay Board finds itself evaluating the financial rewards of all scientists and, because of this, affecting recruitment into science as whole and even the decisions that have to be made (as Drs Gibson and Hawtin point out) years before a young person starts work. Thus a decision consciously to differentiate between the pay that comparable arts and science graduates can command would have repercussions for many years to come. There is no visible sign that the government has decided that it wants a long term decline in numbers going into the scientific profession, indeed it has probably given little thought to the matter, but a settlement that leaves dissatisfaction in the scientific ranks will do much more than lead to temporary discontent.

Finally, and the greatest of the issues raised, does the government know what it wants of its own scientists?

It is obviously attractive to have all those scientific brains at one's disposal but are they always used to advantage? It would be unfair to ask for some sort of deal by which Civil Service science is reviewed in exchange for the coming pay award, but it does seem worthwhile to look again at the whole question of the government offering stable long term employment to such a large number of scientists. There is a feeling that the government holds too many scientists in an undemanding 'parking orbit' and industry and the universities should assume many of the piecemeal roles which the government has acquired. A fairly vigorous policy of hiving off over the next ten years, benevolently supervised by the government, may be no bad thing. And maybe to go with a reduced establishment, greater flexibility could go into pay negotiations. Collective bargaining removes much of the personal involvement that those with highly individual skill should have with their management. It would be good to see a greater devolution of negotiation on to management.

100 years ago



A SUPPLEMENTARY credit of 4,000*l.* has been voted by the Versailles National Assembly for paying a part of the expenses incurred by the observation of the Transit of Venus. Six members belonging to the ultra-clerical party have given a negative vote on a division. It is said they are not believers in the Copernican theory, and have no faith in the astronomical observations.

From *Nature*, 9, 452, April 9, 1874.

Seasonal rainfall forecasting in West Africa

Dr Derek Winstanley, now at the Ecological Systems Research Division, Environment Canada, Ottawa K1A 0H3, emphasises that seasonal rainfall forecasting is vital if early warnings are to be obtained of food shortages.

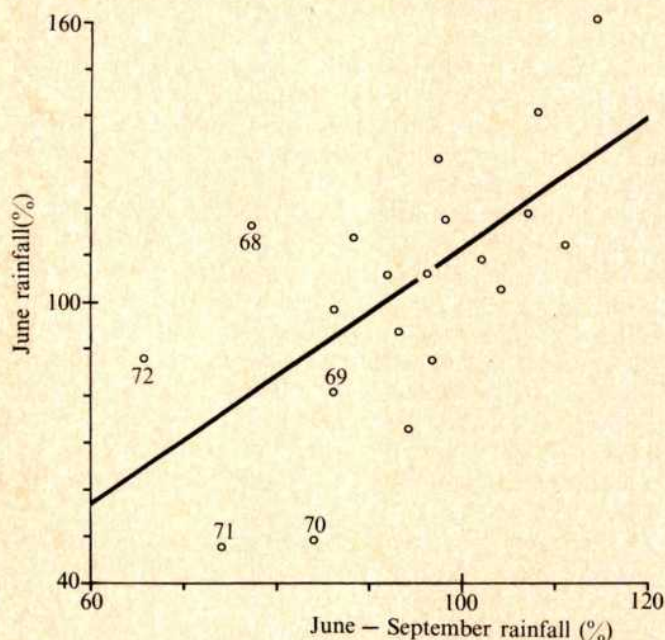


Fig. 1. Mean percentage of normal (1931-60) June rainfall at 59 stations between 10° and 20°N, from the Atlantic coast eastwards to Chad, plotted against the mean percentage of normal June-September rainfall for the 20 years 1953-72. The regression line has been derived by the least-squares method. Data sources are refs 5 and 6

Food production and the maintenance of the ecological balance in the semi-arid zone to the south of the Sahara depend on the rains which fall from June to September. For the past six years the rains have failed¹. Last year 3.5 million head of cattle worth £180 million perished, there was a grain shortage of 1 million tons and some 6 million people were placed on the brink of starvation. In order to avert disaster on an unprecedented scale in 1974, an increase in the already massive amount of international aid is essential. Clearly, successful seasonal rainfall forecasting could lead to an early assessment of food shortages.

I have taken 59 stations between 10° and 20°N, from the Atlantic coast to Chad, and have calculated the mean percentage of normal (1931-60) rainfall for June and for the wet season June-September each year during the period 1953-72. The mean June rainfall for the 59 stations is 124 mm and the mean amount for June-September is 636 mm, 88% of the mean annual total.

Figure 1 shows a highly significant correlation ($r = +0.63$) between June rainfall and the seasonal total. When June rainfall is below normal the total seasonal rainfall is also below normal.

There is clearly a strong tendency for anomalous rainfall patterns to persist throughout the wet season. This suggests an equally strong persistence of the large scale atmospheric circulation systems which control rainfall. The year 1968 was rather anomalous in that June rainfall was 17% above normal but the seasonal total was 23% below normal. This

was the first year of widespread rainfall deficiencies and it is possible that there was a rapid switchover of circulation types in about July of that year. Figure 1 shows that the subsequent dry years fit into the pattern.

The rainfall deficiency in this zone increases with latitude¹: at 18°N there has been a cumulative rainfall deficiency of 250-300% of normal since 1968, whereas at 10°N there has been only a small decrease in rainfall. I have previously suggested that the cause of this decrease in rainfall is a decrease in the northward extent and intensity of the ascending branch of the Hadley cell, concomitant with a weakening of the global atmospheric circulation¹⁻³. Taking the annual number of days of the westerly weather type over the British Isles as an indicator of the strength of the global atmospheric circulation⁴, it is apparent that for the past few years the global circulation has been weaker than at any time for about a century. The drought to the south of the Sahara thus represents an extreme situation but I have suggested that such droughts may become more frequent in the next 60 years³.

It is doubtful whether any nation can cope with a cumulative rainfall deficiency of 300% over 6 years. But the very fact that there are such large scale atmospheric controls on rainfall could provide a rational basis for integrated long term development. What must be determined are the maximum cumulative rainfall deficiencies that can be expected. It must then be decided what level of rainfall deficiencies can be tolerated, if the countries are to maintain some degree of self-support, either nationally or through some political or economic union. Development should then be encouraged in the more favourable areas, even though good rains may extend farther north in some years.

In 1973 world cereal stocks were reported to be at the lowest level for 20 years. Significantly, it was adverse weather in the Soviet Union in 1972 which led to the purchase of huge amounts of American grain; this, probably more than any other single factor, led to the depletion of world grain stocks and the escalation of world food prices. With depleted world cereal stocks it is not easy to guarantee supplies for major disaster relief programmes. Assuming that supplies are available on the international market, the high prices cripple the economies of the drought and flood-affected developing countries; in the case of India, the high cost of food imports has also necessitated the curtailment of the family planning programme.

The main development constraints in this zone are surely climatological, but the task of development seems to be beyond the individual capabilities of the governments. In any future development plans there are three points to be taken into consideration. First, large scale atmospheric circulations exert a unifying influence right across this zone, such that there will again be years when the rains fail over some 5 million square kilometres. Second, the atmospheric controls on rainfall are such that percentage deficiencies, and surpluses, will always increase with latitude. Third, climatic fluctuations in this zone are closely related to climatic fluctuations in other parts of the world, so that in some years there are likely to be synchronous food shortages in different regions which could affect world food stocks.

¹ Winstanley, D., *Bird Study* (in the press).

² Winstanley, D., *Nature*, **243**, 464-465 (1973).

³ Winstanley, D., *Nature*, **245**, 190-194 (1973).

⁴ Lamb, H. H., *Geophys. Mem. No. 116* (Meteorological Office, Bracknell, 1972).

⁵ *World Weather Records* (US Department of Commerce, Washington DC, 1967).

⁶ *Monthly Climatic Data for the World* (Environmental Data Service, NOAA, US Department of Commerce, Washington DC, 1961-72).

international news

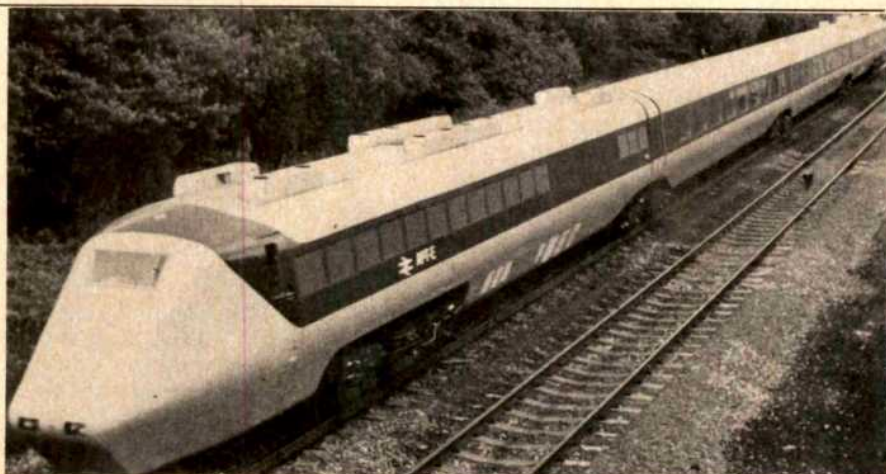
Steel on steel for British rail

John Wilson

BRITISH RAIL (BR) will use steel wheels on steel rails to drive and support intercity passenger trains of the 1980s, travelling at speeds up to 300 km h^{-1} (188 mph). Mr Mike Newman, in charge of BR's Advanced Passenger Train (APT) project, says that no existing linear motor can compete with present conventional electric units in efficiency and believes that the development of a magnetic suspension system with its costly new track is unnecessary for this sort of speed. His opinion conflicts with that of the Commission of the European Communities which recently invited manufacturers of high-power linear motors to Brussels to discuss cooperation in the development of just such a magnetically suspended, linear-motor driven system.

An experimental APT has already started a three year development programme by travelling at 200 km h^{-1} (125 mph) on a test track near the Derby works. This experimental train consists of a power car at each end separated by two trailer cars only, but commercial versions should propel about seven trailer cars. The whole articulated unit is highly streamlined and has a very low cross sectional area, roughly that of Concorde. The final versions will be built as very light semi-monocoque stress skin structures, a technique borrowed from the aircraft industry, and indeed the whole unit resembles an aircraft rather than a train in the design of the interior equipment and accessory systems.

The experimental APT is powered by conventional gas turbines, for the success of the British APT lies not in any revolutionary power plant but in its novel suspension system. Dynamicists at the Railway Technical Centre in Derby were the first to work out the complex mathematics of the ordinary railway wheelset (two flanged wheels rigidly attached to a live axle). They discovered that by altering the cone angles and tyre profiles of the wheels they could make the set roll with a minimum of oscillation. This allows the APT to be run at speeds of up to 250 km h^{-1} (155 mph) even on existing track and to negotiate main line curves



British Rail's APT: running on the same lines into the eighties

50% faster than conventional trains.

Greater speeds than these are possible with APT variants using more powerful engines, but considerable modification of the track is necessary. Mr Newman notes, however, that any improvement made in the track for the benefit of the APT would also benefit normal services. This is in contrast to other methods of high-speed travel where the new track is for the exclusive use of the new vehicle.

The keynote for BR's plans for the new train is the utilisation of existing assets. The brakes, for example provide double the usual performance expected for a normal train—and this in a vehicle of less than half the normal weight. For this reason the early APT's due to begin a trial service on the (London to Glasgow) West Coast main line in 1978 should be compatible with existing signalling equipment. Mr. Newman points out that by improving the track alone electric trains already in service could almost match the performance of the APTs. On a route such as London to Glasgow, which an APT with a speed of 250 km h^{-1} (155 mph) could travel in 3 hours 47 min, a conventional train could take as little as 3 hours 50 min—if all the necessary track improvements were made. Although it is worth noting that the APT could then chop another 37 minutes off this new improved route, the point is that these comprehensive track improvements would cost up to ten times more than an APT system.

The APT is an attempt to stretch an essentially Victorian railway system using 1960s technology. Any successor to the APT will have to be a completely new system. This is because the introduction of APTs is expected not only to compete more effectively with motor-

ways and airlines but also to generate considerable trade itself—to the point, in fact, where the system becomes saturated. It is this saturation, expected about the 1980's, not the need for higher speed, which will lead to the necessity to construct new track, and it is at this point that systems using linear motors, hovertrains and the rest begin to seem attractive.

Cancer research runs into trouble

Colin Norman, Washington

A COMMITTEE of biomedical scientists has delivered a stinging attack on a prominent and highly controversial cancer research programme supported by the US National Cancer Institute. The committee's criticisms and complaints cut deep into the philosophy underlying much of the loudly proclaimed federal war on cancer. Because they raise the basic objection that cancer research shouldn't be targeted and managed like a NASA-style operation to land men on the moon.

The research programme in question, the Virus-Cancer Program (which used to be called the Special Virus Cancer Program) is the epitome of the highly managed, centrally directed approach to cancer research. It now disburses more than 40 million dollars a year, entirely in contracts, to universities and industry for studies on cancer viruses and for the development of resources to support such research.

Ever since it was started ten years ago, with the specific approval of Congress, the Virus-Cancer Program (VCP) has been attacked for a variety of sins, chief of which is that it has been directed by a few people who have

amassed considerable power for themselves and that it has taken money away from potentially more productive research efforts.

In response to such complaints, the National Cancer Advisory Board, the body which oversees the work of the National Cancer Institute, set up a committee last year under the chairmanship of Dr Norton Zinder of Rockefeller University to take a look at how the programme is working. The committee turned in its final report to a board meeting in mid March and it said that virtually all the criticisms that have been raised against the VCP have some credence.

The committee's most basic objection is that "ignorance of the mechanism involved in the cancer process is so profound that it is difficult to be certain where to begin, much less to organize a focused attack". In other words, the basic knowledge just isn't available to begin a concentrated, highly directed research programme on cancer viruses, and the committee reckons that the best way to get the basic knowledge is to support a more broadly based research programme in the universities.

When congress passed the National Cancer Act two years ago, which set in train large increases in funding for cancer research, the implicit suggestion was that by shovelling more money into research and planning the effort with operations research techniques, answers would eventually emerge. A central feature of the act was, in fact, that a national cancer programme plan would be drawn up to provide a framework for the whole operation.

Since the committee's chief objection challenges the philosophy behind the Cancer Act, the report has had a surprisingly warm reception from the people who are in charge of the war on cancer. Dr Frank Rauscher jun., the director of the National Cancer Institute, said in an interview, for example, that the committee produced "a damn good report" and that the Cancer Institute is in fact in the process of righting most of the ills which it spotlights.

But the official enthusiasm for the report is not shared by the scientists who are operating the VCP. They sent a memorandum to the National Cancer Advisory Board in January charging that the committee's suggested improvements in the programme are "based on total destruction of the programme, and not on constructive modifications which could readily be implemented." They also charge, in private discussions, that the committee's criticisms were directed at a few individuals in the programme, on a rather personal basis.

In fact, the committee first presented its report to the National Cancer Advisory Board at a meeting last

November, but the board said that it was going to be so negative it ought to make its criticisms more specific and name names. But the report presented last month was unchanged from the earlier version. Asked why it had not been made more specific Dr Zinder said last week that it was meant to be an overview of the programme and he did not think it proper to single out individuals. He added that the members of the committee are willing to stand behind everything they said.

As for the committee's objections to the programme itself, they centre chiefly on the fact that the VCP is managed by a small group of scientists who have considerable power over the selection of contracts and the report suggests that the process by which contracts are reviewed is not very rigorous. In particular, the committee recommends that bidding for contracts should be more competitive, and that they should be reviewed by panels of scientists drawn from outside the National Cancer Institute.

Although it doesn't say it specifically, implicit in the committee's recommendations is that much of the money now distributed by the VCP through contracts should be made available to the academic community to support individual grants for projects suggested by researchers themselves and not by a handful of managers within the National Cancer Institute.

Zinder said last week that all the committee was trying to do is to "open the programme up to the academic community" by getting more outside people on the review committees and making more money available for investigator-initiated research projects.

The VCP scientists' memorandum to the board says, however, that the programme is not such a closed shop as the committee implies. The contracts support more than 2,000 individuals, including 600 professional scientists and 230 postgraduate and postdoctoral students, whose combined output of scientific papers in 1972 totalled 1,183. Moreover, between July 1971 and January 1974, the VCP received 149 unsolicited proposals for research projects and funded 22 of them, so there is a place for investigator-initiated research in the programme.

Be that as it may, several changes are being made within the National Cancer Institute as a whole and the VCP in particular which meet many of the objections raised by the review committee. For a start, the review process for contracts is being changed to allow greater participation by scientists from outside the National Cancer Institute. An overall advisory committee is being established for the VCP, which will consist entirely of outside scientists and which will advise the director of

the programme and the contract managers on the overall direction of the effort. And the committees which review individual contracts are being reconstituted so that outside scientists will be in the majority.

But the most far reaching change is that the National Cancer Institute is exploring a new funding mechanism which Rauscher calls programme grants. These will be broad based grants to university scientists which will not be encumbered by the controls and specific objectives that are attached to contracts. They will be used to support research designed to fill specific knowledge gaps. The idea is that programme grants would be peer reviewed by multidisciplinary study groups and they would run for perhaps three years.

Lamb's unit to the slaughter?

Allan Piper

THE existence of one of the two climatic research establishments in the western world, the Climatic Research Unit (CRU) at the University of East Anglia, is threatened by lack of financial support, at a time when the importance of climatic research is becoming increasingly clear.

Variations of climate cannot be prevented but they can be predicted with increasing reliability. Advance knowledge of an impending catastrophe—a drought, a flood, a severe winter—allows for careful planning which may often save lives and money. The CRU has been in existence for only two years and its director, Professor Hubert Lamb, says that the prospects for climatic research are excellent: "the plums are ripe for the picking, we only need someone to provide a stepladder".

The climatologists at the CRU are working towards the development of an increased understanding of the mechanisms and causes of climatic fluctuations. They have collected extensive data on weather and climatic behaviour going back to about 1200 AD and present day climatic developments are monitored on a global scale, often using observations from satellites. The knowledge acquired is used to supplement the present statistical techniques of ascertaining likely climatic trends and it is hoped that this will lead to increasingly reliable predictions.

Ever since the CRU was established in January 1972, it has had to operate under restrictive financial conditions. Initial grants came from the Nuffield Foundation, Shell International Petroleum, British Petroleum (BP), the Electricity Council and the Central Electricity Generating Board. These grants amount to less than £60,000 spread over five years and although

Professor Lamb considers them to be "very generous indeed", it is apparent that, compared with other research establishments, the unit is working on a sadly inadequate budget. Furthermore, an initially bad situation has been worsened by the effects of inflation, which eventually forced the withdrawal of the support from BP, although the other contributing bodies have been able to provide appropriate incremental adjustments. Now it is clear that by the end of 1975 the funds of the unit will almost certainly be largely exhausted. Even if the present grants are renewed, Professor Lamb can only remain cautiously optimistic about the likelihood of obtaining support from other organisations.

This is rather disappointing because there is a good case for increased financial investment in climatic research. Modern commercial and industrial techniques are geared to utilising to the limit any available resource, such as water, livestock or crops, and production methods are increasingly arranged to maximise profits. It is always likely that such finely adjusted projects will be vulnerable to any shift in climatic behaviour. Established patterns of production may become severely disrupted, often with disastrous consequences. A continuing decline in

rainfall can ruin not only intensive agricultural projects, but also sophisticated technological schemes such as hydroelectric power plants, or industrial processes which may be sensitive to such factors as atmospheric humidity. Results of recent research to assess differences in agricultural yield between a wet and a dry summer in New Zealand indicate that £2 million a year may be lost just on cattle in one small region alone. Investment in climatic research could save much of this: foreknowledge of climatic trends can facilitate advanced administrative planning, allowing otherwise catastrophic situations to be mitigated, if not avoided, by careful preparations of all necessary assistance.

The results which have so far been produced by the CRU, covering the past two years, are encouraging although not entirely accurate. According to Professor Lamb, however, the inaccuracies illustrate the problems of operating with restricted financial resources. Retrospective reappraisal of the data used reveals that the correct information had, in fact, been assimilated but it was misinterpreted and wrongly collated.

The errors arose chiefly because the unit is drastically understaffed, says Professor Lamb, and the consequent

pressures have not allowed for the assimilated data to be used to its best effect. As a result, the preliminary series of seasonal forecasts has had to be discontinued. There is both the room and the necessity for research workers at all levels of experience from graduate students upwards, and there has been no shortage of suitably qualified applicants; unfortunately the present financial stringency precludes any expansion. A ideally qualified, highly regarded applicant was recently refused a senior research post because of the lack of necessary funds.

Professor Lamb's disappointment is hardly surprising. With the unit left with a guaranteed existence of less than two years, he is not finding it easy to negotiate new sources of support although BP are considering the provision of renewed assistance. It is difficult to understand the reluctance of agricultural and industrial organisations—let alone governments—to grant the required funds, particularly in view of the enquiries already received by the CRU. These include requests from an industrial concern for information about the probability of severe winters occurring simultaneously in Europe, the USA, and Japan, and requests for forecasts from farming organisations in Norfolk, France and even Zambia. But Professor Lamb feels, not unreasonably it seems, that it is the international agencies which should provide substantial support for research which is "vital to the needs of mankind at the present time".

The position of the Natural Environment Research Council (NERC) is also interesting. It is the policy of the council to provide funds only for specified research projects; it will not allocate finances to assist in establishing a research group. For this reason the NERC did not provide support when the CRU was founded back in 1972. Today the NERC provides support for a research student at CRU but will not help to establish any new research projects. This policy contrasts markedly with that of the Medical Research Council (MRC) which will provide 'programme grants' to support "the particular situation in which a university has formally decided to develop a specific plan of study, and the Council agrees in the national interest to assist that development in that particular university."

It would be a pity if the existence of CRU were to be placed in jeopardy after such a short existence, at a time when the potentialities of the research work are about to be realised. The research staff have begun to establish liaisons all over the world, and it would be a great disservice to climatic research if these relationships were allowed to collapse.

US nerve gas plan under fire

A BRITISH expert on chemical and biological weapons has blasted the US Army's plans to develop a new generation of nerve gas weapons on the grounds that they will kill attempts to negotiate an international treaty banning production and stockpiling of chemical weapons. Julian Perry Robinson, a member of the Science Policy Research Unit at Sussex University who has been conducting a study of chemical and biological warfare for the Stockholm International Peace Research Institute, delivered his attack on the army's plans at a symposium organised by the American Chemical Society on April 1.

In September last year, the US Army announced that it is planning to produce new, "safe" nerve gas weapons at the Pine Bluff arsenal in Arkansas. The idea behind the weapons, which are called binaries, is that they will consist of two chemical components neither of which is lethal by itself, but which form a highly potent nerve agent when they are mixed together. The two chemicals would be loaded into separate compartments of a shell and allowed to mix only when the shell is safely on its way to the target.

The army is claiming that binary weapons have the advantage that they are safe to store, and transport, and it is planning to replace the immense

stockpiles of conventional nerve agents now stored in bases throughout the United States and abroad with binaries. These stockpiles have generated considerable public alarm, and opposition to the entire chemical weapons programme in the United States. The army is therefore hoping that its new safe weapons will be more acceptable.

Robinson points out, however, that a programme involving the production of binary weapons and the destruction of stockpiles of conventional nerve agents will cost American taxpayers as much as 2,000 million dollars.

But a more basic criticism of the programme is that if the United States now launches a massive new nerve gas programme, it will completely destroy the credibility of US intentions to negotiate seriously for international chemical weapons control. Talks have been taking place in Geneva for several years on a possible treaty banning use and stockpiling of such weapons, and although the United States position has not been exactly enthusiastic for such a treaty, at least it has shown willing to talk. Robinson argues, however, that "a decision to go ahead with binaries would almost certainly mean an end to the disarmament negotiations, and with them a prospect for improving US security to a far greater extent than binaries ever could".

Oil prices hamper oceanography

FOR most users of oil the recent price rises have simply meant paying more for the commodity and finding ways of living with the greater expense. For some, particularly those receiving fixed sums of money from the government, paying more is not possible without going bankrupt. In scientific research the worst affected are the oceanographers.

In Britain the Natural Environment Research Council (NERC) operates many of the research ships for universities and its laboratories. Largest of these are the *Bransfield*, *Discovery*, *Shackleton* and *Challenger*, although there are many smaller vessels some of which are the direct responsibility of individual universities. An official of the NERC pointed out that there would be an increase of "several hundred thousand pounds" in the 1974 fuel bill for these ships, this in a total budget for the operation of research vessels of only £1.5 million. As yet the NERC has not been able to extract any promises of extra money from the Treasury, so although marine operations are for the present carrying on as usual, within a few months there is a serious danger that cruises will have to be curtailed or cancelled.

One oceanographer expressed concern that the short term problems with oil prices should not affect long term decisions on building new oceanographic vessels. "It is clear that large scale capital expenditures are very vulnerable at times like these", he said, "but we cannot indefinitely postpone the renewal, let alone the expansion, of our oceanographic fleet". At present there are no new ships planned but there seems to be a general feeling both that some vessels cannot last much longer and that oceanography actually needs more ships to satisfy the demands of research workers. It looks, however, as if survival at the present level of activity is going to be the most that the NERC can aim for in the next year or two.

Soviet reactor accident: official

from our Soviet Correspondent

REPORTS of a serious accident to the BN-350 fast breeder reactor at Shevchenko on the Caspian Sea, although strenuously denied by official Soviet resources, do seem to have some foundation in fact, although the failures involved belong to the field of conventional, rather than nuclear, engineering.

According to Dr N. V. Krasnoyarov, interviewed when in London for an international conference on fast reactor power stations, three out of the six

generators of the Shevchenko station have, in fact, been taken out of service following faults in the interface of the secondary (sodium) and tertiary (water) circuits. In one case, the leakage of water into the sodium of the second circuit was observed at a very early stage and the generator was immediately closed down; in the other two cases, the water, reacting on the hot sodium with consequent liberation of hydrogen, caused a pressure buildup which resulted in the rupture of what Dr Krasnoyarov called "special safety membranes" (presumably bursting disks); the products of the reaction were conveyed to a special dump tank and the generators concerned were closed down.

All three faulty generators are now undergoing repair; in the meantime, the other three are being operated at below nominal capacity, giving a total output at the station of some 30% of its rated power. The deficit of power required for electricity generation and desalination is at present being supplied by the conventional oil-fired generators which the BN-350 was designed to replace but which were retained as a peak-demand backup. The primary (radioactive) sodium circuits were in no way involved.

The rumours that more serious damage had been detected by United States surveillance satellites seem to have grown by a snowball effect, from 'If

there had been a serious accident, the satellites would have detected it' to 'The satellites have detected a serious accident', and the July dating seems to have crept in by confusion with the date of commissioning of the station. The growth of the story was in no way quenched by the tone of the official denials. According to the Novosti agency, Academician Andranik Petrosyants, Chairman of the State Committee for Atomic Energy of the Soviet Union "emphatically denied" that anything untoward had occurred. Instead of the reasonable explanation offered by Dr Krasnoyarov, Academician Petrosyants simply maintained that "all this information does not correspond to reality" and that the report "is an invention and obviously pursues some other aims", being directed against Franco-Soviet cooperation in fast breeder reactors. "It is evidently of benefit to some people to try to do damage to that cooperation and set public opinion against that cooperation by inventing 'explosions'."

The tone of the denial seems inspired by considerations of prestige, rather than a desire to impart information. Perhaps if Academician Petrosyants had, in the name of the "cooperation" he advocates, been a little more forthcoming about what are after all relatively minor faults, his denial of a major disaster might have been found more credible.

Better deal for polytechnics

THE 30 polytechnics in Britain are to get a better deal from the Science Research Council (SRC) as far as postgraduate studentships are concerned but the criteria governing the award of research grants will still favour university-type research. This move follows the report of the SRC's Polytechnics Working Group which has been considering the relationship between the SRC and the polytechnics since 1972.

The SRC is to put its new plans into operation through a new Committee on Postgraduate Training in the Polytechnics, which has been set up for a three-year period under the watchful eye of Dr A. H. Chilver, Vice-Chancellor of the Cranfield Institute of Technology. The Committee will be empowered to award advanced course studentships (usually lasting one year and aimed at the taught postgraduate course which the polytechnics have been encouraged to set up).

As to the award of research studentships, usually held for three years, the new committee will only advise other parts of the SRC "on any special factors to be taken into account in the award of research studentships to polytechnics". The SRC is keen that this should result in the channelling of more of

the so-called CASE awards (Cooperative Awards in Science and Engineering) to polytechnics, thus giving a fillip to the cooperation between polytechnics and industry which the Department of Education and Science has always been keen on. At the end of 1972 students in polytechnics held 1.7% (104) of all SRC research studentships and 2.9% (45) of its advanced course studentships.

The volume of research in polytechnics is bound to increase in the next few years because by 1981 the government plans that there should be 180,000 undergraduate and postgraduate students in polytechnics on full-time or sandwich courses, compared with 68,000 in 1972. Clearly there will be a big demand for whatever the SRC has on offer.

Dr J. S. Bosworth, Director of Newcastle-upon-Tyne Polytechnic, said last week that the SRC's plans are certainly a good step in the right direction. He pointed out, however, that it is particularly important for polytechnics to get together with other interested parties and define just what research they are to do. In this way, he said, it should be possible to arrange a framework in which polytechnic-type research could be sensibly planned and carried out.

Lead in the environment

A RECENT report of rising levels of lead in the blood of people living near a large British motorway interchange has highlighted the problem of lead pollution through exhaust emission. Lead can enter the environment in many ways. Food is generally considered to be the main source of lead intake; it has been estimated that each person takes in about 200–250 micrograms of lead in food daily. Lead is also released during lead smelting and other industrial processes but these are now subject to strict controls and although there is still pollution this affects only small areas.

In 1972, about 90,000 tonnes of lead were added to petrol in the United Kingdom alone. Plans to reduce the level of lead in petrol to 0.45 grams per litre by 1975 have had to be shelved in view of the present oil crisis as producing high octane grades of petrol without the use of lead additives needs much more crude oil. At present the permitted maximum in the United Kingdom is 0.64 grams per litre.

The survey of blood lead levels of adults and children living around the Gravelly Hill interchange, 'Spaghetti Junction', was carried out by Birmingham City Council. Before the interchange opened in 1972 lead levels in residents' blood were on average 12.2 micrograms per 100 millilitres and last October had risen to an average of 16.6 micrograms. This January they showed a rise to an average level of 21.0 micrograms by the hospital analysis and 26.3 according to the Birmingham City Analyst. These discrepancies highlight one of the main problems in lead toxicology; the variability inherent in the methods used to measure blood lead levels.

The inference that is being drawn from the Birmingham report is that lead from traffic exhaust fumes is the cause of this rise in blood levels. The Birmingham Health Committee have expressed concern and are drawing the government's attention to the report. Although the average levels do not yet approach the accepted toxicity levels and are not above the upper limits for normality, a great deal of concern has been expressed over the past few years about the long term effects of exposure to subtoxic levels of lead especially in children. Also, there are bound to be individual higher than average levels.

The maximum acceptable level of lead in the blood of adult male workers exposed to lead for an eight hour day is considered to be 80 micrograms per 100 millilitres. For children the general consensus of opinion takes 36–40 micrograms as the upper limits of nor-

malinity and considers 60 micrograms as cause for concern even though the child may not be showing any symptoms.

The whole question of the relation between the amount of lead in the atmosphere and its absorption into the body and link with blood lead levels is under intensive study at present. A study in 1972 by the Medical Research Council's Air Pollution Unit found that atmospheric levels of lead had risen in a busy London street from an average of 3.2 micrograms per cubic metre of air to 5.4 micrograms. A small survey of London taxi drivers during this time compared the blood lead levels of daytime with night-time drivers. Although



Spaghetti Junction: children at risk?

the daytime drivers were subjected to a higher level of exhaust pollution (monitored by the carbon monoxide effect on the blood) their blood lead levels were similar.

Much work on the size and aerodynamic properties of the lead particles given out in exhaust fumes is in progress. It has been estimated that about one-quarter of the lead content of petrol remains in the engine and the rest is given out in the exhaust as fine particles of lead compounds. Of this about half is thought to be deposited within a few hundred feet of the road but the finest particles may be carried considerable distances in the air. The fate of these particles in the lungs and how much is finally absorbed is also under study. The Atomic Energy Research Establishment at Harwell have just embarked on a study of the intake of radiolabelled lead from exhaust fumes to try and throw light on this problem. Earlier work on artificial aerosol lead intake indicated an uptake of about 40–50%, and more recent

work in other laboratories suggests that about 15–20% of atmospheric lead is absorbed. Some workers, however, think that this figure is too low.

At present, there are many surveys measuring atmospheric and blood levels but it is often difficult to correlate the results of such different surveys. In a recent report, the Central Unit on Environmental Pollution drew attention to the difficulty of correlating results from different surveys. The report names lead as one of six pollutants which need monitoring on a wider scale than before. They recommend that a network of 20 sites be established in various areas to monitor trace elements by a standard technique. At present, a survey of traffic fume pollution is underway in five major British cities, coordinated by the government's Warren Spring Laboratory.

Additionally, the Worshipful Company of Pewterers celebrated its 500th anniversary by setting up a trust fund to provide an income of £10,000 a year for a research group at the Institute of Neurology, enquiring into the effects of heavy metals on the central nervous system. The first Pewterers Fellow, Dr Jeremy Barlow, has pointed out that, for the time being, the group doesn't quite know what it's looking for, but that there are certain facts, like the increased incidence of mental retardation in children in recent years, and about the amount of lead in the environment, which may or may not be related to each other.

The situation is further complicated by the fact that some individuals have a greater tolerance of lead in the body than others; one man may have taken in lead to the extent that his blood contains 1,000 micrograms per millilitre, and appear to be no worse off for the experience, whereas another individual may exhibit the clinical symptoms of lead poisoning at levels which are accepted as relatively safe. What's more, the symptoms are easily confused with the general symptoms of what is known as 'modern living' — gut pains, insomnia, loss of appetite, and headaches, so that it's difficult to say that lead is specifically responsible whenever these symptoms occur.

Clearly a great deal of research has yet to be done before it is possible to say whether or not atmospheric pollution from car exhaust fumes is in any way responsible for higher levels of lead in the body, and that life, or health is endangered by the motor car. If we really want to be on the safe side in the meantime, it's possible to cut the lead content of petrol by producing high octane grades at great expense, or to stop using high-compression engines, which would mean that an awful lot of nice new cars would be made obsolete, at a stroke.

correspondence

Prices of scientific journals

SIR,—The risk of being misquoted from telephonic communications (me to *Times Higher Educational Supplement*, October 26, 1973) increase when the misquotation is quoted, for I am the Alec Henderson who figures in John Hall's article (*Nature*, February 15). One original misquotation was that the well known *IEEE Transactions* becomes the mythical *Transactions of the American Association of Electrical and Electronic Engineers*: but the desolating fact is that *IEEE Trans.*, having already gone up from £252 to £321 this year, has now gone up again to £411—a rise of 63% in a year. On the other hand, it is fair to point out that the quoted increase in price in *Current Contents: Life Sciences* applied only if one took a new and optional indexing service.

The rate of rise in journal prices is alarming. That of books has hitherto been somewhat less but some alarming trends are evident, particularly in marking up of in-print books in publishers' stocks. Recent correspondence with the American publisher of a work on polymerisation explained that the rise in the United Kingdom price of a book of 416 pages from around £16 to £24 was due to floating exchange rates, air-freight charges, warehousing and distribution costs and paper costs—all in the case of a book first published in 1972, not reprinted and listed at the original price in the publisher's catalogue dated 1973–74.

University library budgets in the United Kingdom as a whole were not keeping up with the combined effects of rising prices, diversification within universities and proliferation of literature even before Mr Barber's cuts in public spending. To these cuts, particularly to that in supplementation for price increases, they are particularly vulnerable because of the large proportion of their expenditure which goes on books and journals and subacademic staff (supplementation of academic salaries being safeguarded): they must therefore receive generous treatment from their universities if they are not to fall further behind in service to their readers. Librarians thus do not need to be urged by Professor Linnett and his co-signatories to "exercise the greatest reticence on subscriptions to new journals"; they already have to do so,

against the insistence of their scientists (and others) that exception must be made for the latest expensive journal which is just their thing.

If scientists can reduce the volume and cost of publication, whether by refraining from publishing or by compression, they will themselves reap the benefit of the greater accessibility of what is published since their libraries will be able to afford more of the available literature. If Mr Maxwell's plans for *Tetrahedron* will reduce by 80% its price (which I do not suggest is excessive) as well as its bulk, Bubb, Humphreys, Anderson and their fellow librarians will be glad to join with Finniston, Hartnett, Kurti, Thompson and their fellow scientists in praising his "imaginative leadership".

ALEX. ANDERSON

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SIR,—The best way through the scientific prices jungle (*Nature*, 247, 417; 1974) is for scientists to give wholehearted support to librarians in their efforts to establish objective criteria of usefulness. It is possible to tell how frequently a title is cited in published papers (especially since the "Journal ranking package" has become available from the Institute for Scientific Information); it is possible to tell how often the title is photocopied, how often the title is borrowed on inter-library loan and how often it is being consulted in the library, if each volume and part has a consultation slip. These may not be very good measures of usefulness but they are much better than no measures at all. All that is lacking is a firm decision on the part of the scientific community that:

- No journal should be bought until the cost of borrowing it from the British Lending Library exceeds the cost of buying and storing it on site.
- No journal held on site should be maintained unless objective evidence can be produced that it is being used.

I have little doubt that this policy, if strongly backed, would be a major factor both in improving the quality of science journals and in controlling the prices jungle, to the benefit of library users and librarians alike.

P. G. PEACOCK

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Government scientists' pay

SIR,—It is now widely known that government scientists have been in dispute with the Civil Service Department for over three years because the department has been unwilling to agree on any reasonable criteria for determining the level of scientists' pay. As a result of this long and bitter dispute, government scientists have seen their pay fall, relative to colleagues with whom previously they enjoyed parity, by 20% and in some cases by even more. Since the government is the major employer of scientists in the United Kingdom, this decline in the pay of government scientists has led to the whole of the science area becoming a depressed industry.

What does not seem to be so widely appreciated is the likely long term effect. The status and success of the United Kingdom in the world have been founded to a large degree on the inventiveness of its people and their achievements in the field of science and technology. What is true of our history seems to us even more true today. Yet, at this critical time, we find that 6,000 places for scientists in the universities are vacant, that fewer young people are studying science at school and that scientists are leaving their chosen careers at an alarming rate to take up other occupations.

Since a decision to adopt a scientific career is often taken at the age of 15, in effect it takes six years to train a graduate and nine to train a PhD. Thus the ultimate effect will be to damage the growth of all science-based industries for a decade or more. We believe this is too high a price to pay for an unnecessary dispute.

As responsible members of the scientific community, we look forward to a speedy resolution of the present dispute and to a time when it will again be possible to urge young people to take up science as a socially desirable and reasonably rewarded profession.

J. A. GIBSON
P. HAWTIN

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news and views

Chinese granites and rapid seafloor spreading

THE attention of geologists and geophysicists, for a long time focused on land masses, switched to the oceans in the mid-nineteen sixties when it became clear that the movement of continents could only be understood in terms of processes occurring in ocean basins. Now that there is a fair consensus on submarine geology and geophysics, attention is switching back to continents where there are still some very difficult problems to solve but where the insights of the past ten years can help. Two new papers exemplify the questions that are being asked.

Plate tectonics is a good framework for running the geological clock backwards for the most recent 100 million years, as the magnetic record is well preserved for this period in oceanic crust. Attempts to deduce the disposition of the continents at earlier dates, however, can be less certain because much of the ocean crust formed then has been consumed at island arcs. As with Watergate, the magnetic tape recording is missing, so other sources of evidence must be sought on land. This Jahn does later in this week's *Nature* in using newly determined ages of rocks in south-east China to study a period of exceptionally vigorous seafloor spreading about 100 million years ago. Correlations which he tries to establish within the time of magnetic records may then be extended to earlier times of intensive granitisation when there is no seafloor record.

By a coincidence it is the same area which is the subject of the other paper which originated in the Department of Geology, Nanking University (*Scientia sin.*, 17, 55-72; 1974). Better knowledge of tectonics means better knowledge of mineral deposits. The cycles of tectonic activity in south-east China have led to rich sources of ore, and it is of great importance to know which regions are richest and thus can be most easily exploited.

The Nanking group have dated the distinct tectonic zones of granites that run NE-SW in south-east China. In general the older the rocks the further inland they are. Each zone is about 100 km wide and characteristic ages are 900, 480, 200 and 100 million years, the last being called the Yenshan tectonic zone and forming the coastal belt. The authors indicate the following series of events for each cycle of tectonism: subsidence, sedimentation often with volcanism, orogenic movements, granitisation, regional uplift, subsequent crustal movement and small scale granitisation. They go on to observe "the further development of the Himalayan orogenic zone in our Taiwan province and the presence of island arcs and a submarine trench east of Taiwan indicate that the same tectonic processes have been going on at present".

Analysis of metallogenetic relations and mineralisation capacity of the granite of different zones necessarily produces complex results, but there are some very striking unifying factors. They are best seen in multiple-aged composite granite bodies where several tectonic cycles have left their mark. Ore forming elements such as beryllium, tungsten, tin and niobium are progressively concentrated in the younger bodies. Yenshan rocks contain beryllium, niobium,

tantalum and scandium in workable deposits. The authors conclude that it is the recurrence of granitisation rather than its occurrence on one occasion which leads to significant metallic deposits.

But what are these cyclic tectonic events? It is this question to which Jahn's paper contributes, as it looks at the Yenshan orogeny. Jahn is limited to samples from the offshore islands; these give ages of around 100 million years. Thus the Yenshan orogeny occurs simultaneously with a postulated time of very rapid (up to five times normal) seafloor spreading, established from marine magnetic data. What is more, many others have observed vigorous activity around this period in circum-Pacific regions. Maybe there is a correlation between rapid spreading and intensity of thermal episodes, caused perhaps by an enormous increase in viscous heating as old plate descends into the upper mantle. Jahn is cautious about such a correlation, but the evidence is certainly persuasive.

Some interesting problems are raised by these two papers. If this correlation is correct, is it generally correct to associate all previous Chinese orogenies with times of rapid seafloor spreading (for which it has been already noted there is no magnetic record)? Was rapid spreading a world-wide phenomenon, and how was it caused? Will the next pulse of rapid spreading convert the continental shelf off China into another tectonic belt? These are good questions for bright research students.

D.D.

Ghost neutrinos emerge from the mathematics

IT is a remarkable fact that even after two generations, the investigation of solutions to Einstein's famous field equations of general relativity is still very much in its infancy. There are indeed very few exact solutions available, and some of those which are have very bizarre properties. One recent solution, published by Davis and Ray of Clemson University in *Physical Review D* (9, 331; 1974) is a distinctive mathematical novelty.

To understand the implications of this new result, first recall that the gravitational field equations of general relativity are a prescription for calculating the curvature of space-time from the mass-energy content of the Universe; that is, the equations relate the geometry of space-time to the physics of matter and energy. Thus for a given distribution of matter say, the geometry of space-time in its vicinity may in principle be deduced. In practice, the equations are prohibitively difficult to solve in all but the simplest cases. One of the earliest solutions, due to Schwarzschild, give the geometry around a spherically symmetric object such as a star. The gravitational bending of light by the Sun testifies to the curved nature of space-time in its vicinity and Schwarzschild's solution gives the correct amount of curvature. Just as the geometry is determined by the material content of space, so in turn the behaviour of mass-energy depends on the geometry. There is clearly a problem of self consistency here.

Among the more elusive of the elementary particles is the neutrino, the particle produced in, among other circumstances, the β decay of a radioactive nucleus. Neutrinos have no mass or charge, but they do carry spin, and are

rather like massless, chargeless electrons. They interact only very weakly with ordinary matter so that most would pass right through the Earth without stopping. Nevertheless, neutrinos cannot be ignored in general relativity, for it is widely believed that they are the most numerous of all particles in the Universe, having emerged in copious quantities out of the 'big bang'. The motion of the neutrinos, which is always at the speed of light, is described by the Dirac equation. A self-consistent solution of the combined Einstein-Dirac equation therefore supplies a possible space-time geometry consistent with a possible current of neutrinos. It is just such a solution which Davis and Ray have found, but one with rather strange properties.

The precise mathematical quantity which describes the curvature of space-time is known as the Ricci tensor $R_{\mu\nu}$, which is a 4×4 array of numbers defined at each point of space-time. Davis and Ray choose a geometry which is restricted partly by having plane symmetry around the x axis and it turns out that for this case only the diagonal components $R_{\mu\mu}$ of the array are nonzero. On the other hand, the physical quantity which curves space-time is the stress-energy, which may also be written as a 4×4 array of numbers at each point. Using the Dirac equation in their selected geometry, Davis and Ray then proceed to calculate this second tensor for the neutrinos. All but two of the components are immediately seen to be zero and of the two that are nonzero neither is along the diagonal. Now in the neutrino case Einstein's field equations simply reduce to the equality of these two tensors (apart from a multiplicative constant including Newton's constant of gravitation, G). Consequently, the two remaining off-diagonal terms of the stress-energy tensor must also be zero, because all the off-diagonal $R_{\mu\nu}$ terms are. So it turns out that the whole stress-energy tensor for the neutrinos in this particular space-time vanishes. Einstein's equations are exactly the same as they would be for empty space. And in fact, these very empty space equations were already solved many years ago by Taub.

At first sight it might seem that Davis and Ray had somehow put the neutrinos in and then taken them out again. This is, however, not so. That the neutrinos are still there is demonstrated by the fact that the neutrino current is explicitly nonzero. In principle it would be possible to detect such a current in a β -decay experiment.

Of course there is no suggestion that the solution described here in any way corresponds to the situation actually found in the real Universe. Nevertheless, it is somewhat surprising to find that the general theory of relativity admits at all the possibility of a current of particles which may produce physical effects, and yet make no additional contribution to the gravitational field. Because of this property Davis and Ray have invented the term 'ghost neutrinos'. It would be interesting to know if ghost particles may exist under a wider range of circumstances.

P.C.W.D.

Amplification of VLF radio signals

VERY low frequency (VLF) radio waves have several interesting properties. In the ionosphere they split into two waves; one is strongly reflected and is of immense importance for long distance communications, the other, the extraordinary or 'whistler' wave, travels through the magnetosphere weakly guided by the Earth's magnetic field.

Whistlers, from which this type of propagation gets its name, were probably first detected on telephone lines in Austria towards the end of the last century, but the first published examples were those observed during the First World War by Barkhausen, a German radio engineer,

as he listened in on allied telephone conversations.

The whistler is a falling tone, the frequencies (f) of which Eckersley found to have travel times which were proportional to $1/\sqrt{f}$. It was not until the early 1950s that Storey, working in Ratcliffe's group at Cambridge, discovered that whistlers were in fact the dispersed radio energy from atmospherics generated in thunder storms. Storey showed theoretically that the VLF electromagnetic energy would propagate along the Earth's magnetic field direction and be received in the opposite hemisphere as a falling tone with the right frequency-time characteristics. The whistlers which were received at Cambridge travelled along paths which carried them to more than 10,000 km from the Earth and showed, for the first time, that at this distance there were an appreciable number of electrons and ions ($\sim 500 \text{ cm}^{-3}$) whereas, formerly it had been considered virtually void.

This discovery triggered off considerable research activity and the number of natural VLF phenomena was multiplied; efforts to explain them lead to a greater understanding of the plasma and energetic particles trapped in the Earth's magnetic field. Many of the generation mechanisms proposed for natural emissions involve growth or amplification of electromagnetic wave by means of resonant interaction with energetic particles. A recent experiment carried out by McPherson, Koons and Dazey of the Aerospace Corporation and by Dowden, Amon and Thomson of the University of Otago (see later in this issue) has revealed that the signal from ground transmitters may also be amplified in the magnetospheric plasma. A signal at 6.8 kHz from a transmitter in Alaska was received in New Zealand at a station which was situated close to the geomagnetically conjugate point. The interesting feature of the experiment was that during the transmission the received signal strength increased, for a short period, from below noise level to a maximum of $35 \mu\text{V m}^{-1}$, before fading again. This maximum value represented a signal 27 dB greater than that calculated theoretically and the authors attribute this to amplification in the magnetosphere.

One of the chief difficulties in this type of experiment is to radiate adequate power at these long wavelengths ($\sim 200 \text{ km}$) and this was neatly overcome by these experimenters by using a monopole held vertical by a balloon at an altitude of between 1 and 1.5 km. Even with this arrangement the total radiated power was only 13 W.

It is interesting to note that McNeill, also in New Zealand, reported (*J. geophys. Res.*, 73, 6860) as early as 1968 evidence of amplification of signals from the Seattle transmitter (NPG) at the rather higher frequency of 18.6 kHz. This effect, which amounted to an amplification of about 10 dB, was noted by McNeill during studies of frequency shifting mechanisms in the magnetosphere.

It is now generally agreed that to receive VLF signals in the conjugate region the energy must be guided by ducts of enhanced ionisation (or perhaps troughs). It seems from work carried out mainly at Stanford that in the absence of such enhancements the wave energy is not guided sufficiently closely to the Earth's magnetic field to produce an appreciable signal in the opposite hemisphere and in some cases the energy may even be reflected or absorbed. McPherson *et al.* and Dowden *et al.* identify the duct in which the propagation probably occurred from whistlers which were observed during the transmission period. All the whistlers observed were shown, by a now well established technique, to have travelled in a single duct and the authors quite reasonably assumed that the energy from their transmitter travelled in the same duct.

Though this experiment was performed only at 6.8 kHz, the transmitter is capable of working at frequencies up to 21 kHz and future research should show how this amplification depends on frequency, the time of day, transmitted power and the conditions prevailing in the magnetosphere.

Another amplification or wave growth process which has received attention recently is the transverse resonance instability. In this instability, which leads to the generation and growth of VLF waves in the magnetosphere, the high energy tail of the trapped particle distribution is unstable if the pitch angle distribution is anisotropic. The process is thought to explain ELF and VLF hiss at medium latitudes. Kennel and Petschek (*J. geophys. Res.*, **72**, 1; 1966) originally showed that particles with energies in excess of the magnetic energy for particles ($B^2/8\pi N$, where B is the magnetic field strength and N is the ambient plasma density) would be unstable to wave growth and that the instability would lead to particle precipitation and a limit to the fluxes of particles trapped in the magnetosphere. This limit to the numbers of trapped particles was considered important and in agreement with satellite observations. The existence of this limit is now questioned by Etcheto and her colleagues (*J. geophys. Res.*, **78**, 8150; 1973) who have established a self-consistent solution to the equations governing the wave growth and particle diffusion. This solution suggests that there is strictly no 'limiting flux' and that the equilibrium flux of trapped particles depends on the rate of injection of particles into the trapping region of the magnetosphere.

The amplifying and wave growth processes are clearly of increasing importance in this field for they contribute in different ways to both the strength of the transmitted whistler mode signal and the background noise which interferes with its reception.

A.R.W.H.

Aspirin, Boston and Little Rock

from a Correspondent

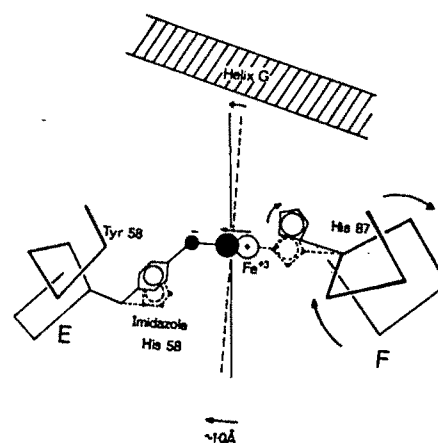
IT may be asserted with some confidence that the genetically determined haemoglobin variants provide the most impressive roll-call of place names from all over the world, from Saskatoon to Sydney, and from Toronto to Tongariki. The appearance of aspirin in the title of this column, however, calls initial attention to a paper which appears to dispose of yet another proposed therapy for sickle cell anaemia. Starting from the established transfer, both *in vitro* and *in vivo*, of the acetyl group of aspirin to an ϵ -NH₂ group of lysine of serum albumin, Klotz and Tamm in 1973 had reported a similar transfer to haemoglobin, accompanied by a substantial increase in oxygen affinity. It was considered that this shift might be due to acetylation of the NH₂ terminal valines; a similar shift is observed following their carbamyla-

tion by cyanate, which is being intensively studied as a possible drug for the inhibition of sickling of sickle-cell anaemia red cells *in vivo* (see *Nature new Biol.*, **242**, 33; 1973).

De Furia, Cerami, Bunn, Lu and Peterson (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3707; 1973) have re-examined the effect of aspirin on the oxygen affinity of haemoglobin. They find that although the acetyl group is indeed transferred, both *in vitro* and *in vivo*, there is no effect on the oxygen equilibrium of haemoglobin, either in solution or in intact red cells. Furthermore, patients on long-term aspirin therapy show no shift in the oxygen affinity of their blood. They also failed to observe any inhibition of *in vitro* sickling of deoxygenated sickle cell erythrocytes previously incubated with aspirin, and conclude that this drug is of no value in the treatment of sickle cell disease.

The gelation of sickle cell haemoglobin itself has been the subject of theoretical study. Minton (*J. molec. Biol.*, **82**, 483; 1974) has devised a thermodynamic model to account for the macroscopic solution properties of Hb-S in terms of microscopic structure and interactions. In this model step 1, the formation of a rod-like microtubular array, is treated as being equivalent to a precipitation. Step 2, the formation of the nematic gel phase, is treated as an isotropically-aligned transition in a suspension of interacting rod-like particles. By combining the two steps a qualitative temperature-composition phase diagram is obtained which provides a unified interpretation of many of the observed properties of Hb-S solutions.

In Hb-M(Boston) the distal histidines in the haem pockets of the α subunits are replaced by tyrosines; His E7(58) \rightarrow Tyr. The iron atoms of the abnormal α subunits remain in the ferric state to give a natural valency hybrid α_2^{+M} Boston β_2^{deoxy} . In heterozygotes for Hb-M(Boston) there is methaemoglobinemia because of the ferric α chains and cyanosis because of the low oxygen affinity of the normal β chains. Pulsinelli, Perutz and Nagel (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3870; 1973) have now studied this variant by X-ray analysis. A difference electron density map of deoxy Hb-M(Boston) minus deoxy Hb-A shows that the ferric iron atom in the α subunit coordinated to the four nitrogens of the porphyrin and the phenolate group of the tyrosine. The normal bond to the proximal histidine F8 (87) is absent and the iron, which is therefore five-coordinate, lies on the distal side of the porphyrin ring (see figure). The rupture of this bond causes the F helix to shorten slightly "like a spring released from a tension that had kept it slightly un-



Stereochemical changes in the haem pocket of the α subunit on going from deoxy Hb to Hb-M(Boston) (from Pulsinelli *et al.*).

coiled". The resulting changes in tertiary structure of the α subunits stabilizes the deoxy or T structure of the haemoglobin tetramer, which lowers the oxygen affinity of the normal β subunits. Thus the clinical effects and many of the abnormal properties of Hb-M(Boston) can now be accounted for in structural terms.

Haemoglobin Little Rock is a high oxygen affinity variant which is unique in having normal Bohr effect and haem-haem interactions. Collaboration between Bare, Alben and Bromberg (Ohio), Jones and Brimhall (Oregon) and Padilla (Little Rock) (*J. biol. Chem.*, **249**, 773; 1974), has now established that the alteration in sequence is β His H21 (143) \rightarrow Gln. Since the β 143 histidine is an important binding site for the allosteric effector 2, 3-diphosphoglycerate, they have studied the effect of DPG on the oxygen affinity of Hb-Little Rock. They find that the DPG dissociation constant for deoxy Hb-Little Rock is about twice that for deoxy Hb-A, and the corresponding value for the oxy form about four times that of oxy Hb-A. They conclude, however, that the higher oxygen affinity of Hb-Little Rock cannot be explained solely in terms of reduced allosteric interaction with DPG, and that the Gln residues at β H21(143) must have a further specific effect on the oxygenation equilibrium.

Immunodepression in protozoan infection

from our Parasitology Correspondent

ANY ideas that patients with malaria or sleeping sickness readily succumb to other infections because of malnutrition associated with the disease are losing ground as more evidence accumulates to suggest that the immune response to other antigens is depressed

during protozoan infections. As long ago as 1962 it was realised that children suffering from malaria had diminished responses to tetanus toxoid (McGregor and Barr, *Trans. R. Soc. Trop. Med. Hyg.*, 56, 364; 1962) but the reasons for this were not investigated for another decade. Under experimental conditions, mice infected with malaria respond less well to tetanus toxoid than do controls (Voller, Gall and Manawadu, *Z. Tropenmed. Parasit.*, 23, 152; 1972) and this has been confirmed in children (Greenwood *et al.*, *Lancet*, i, 169; 1972). The mouse malaria model has provided considerable information on immunodepression during malaria infections and the antigens to which the immune response is diminished include not only standard preparations but also infectious agents such as Moloney leukaemia virus (Bamford and Wedderburn, *Nature*, 242, 471; 1973).

In laboratory models of trypanosomiasis, Goodwin and his colleagues (*Brit. J. exp. Path.*, 53, 40; 1972) found that infected mice and rabbits had reduced abilities to produce antibodies against sheep red blood cells and Urquhart and his associates (*Trans. R. Soc. Trop. Hyg.*, 66, 342; 1972) found that the rejection of the nematode worm *Nippostrongylus brasiliensis* was impaired in rats with trypanosomiasis. Immunodepression is less easy to test in human trypanosomiasis, but Greenwood, Whittle and Molyneux (*Trans. R. Soc. Trop. Med. Hyg.*, 67, 846; 1973) were able to examine 38 patients with Gambian sleeping sickness using skin tests antigens and *Salmonella typhi* vaccine. The humoral and cellular responses were both less in these patients than in 43 uninfected controls. None of the patients was suffering from malnutrition.

Immunodepression has also been shown to be associated with experimental toxoplasmosis by Strickland and his associates (*J. infect. Dis.*, 126, 54; 1972). Infected mice were less able to mount an immune response against malaria than controls.

A general pattern seems to be emerging in which immunodepression is a constant feature of protozoan infections. The effects need not necessarily always be harmful for autoimmune diseases may be prevented in infected animals as in the cases of allergic encephalomyelitis and trypanosomiasis (Allt, *et al.*, *Nature*, 223, 197; 1971) and spontaneous autoimmune disease of New Zealand mice and malaria (Greenwood and Voller, *Clin. exp. Immunol.*, 7, 805; 1970).

The mechanisms of immunodepression are still obscure despite the wealth of examples available for study. Antigenic competition is thought to be one explanation, but the possibility of

specific interference with the function of T cells is receiving attention (Bamford and Wedderburn, *Nature*, 242, 471; 1973). The problem deserves urgent attention because of the possible danger involved in vaccinating children and others infected with a range of tropical diseases. The relations between malaria and Burkitt's lymphoma have already received considerable attention (Ziegler, *et al.*, *Trans. R. Soc. Trop. Med. Hyg.*, 66, 285; 1972).

A curious difference between parasitologists and virologists exists in this field. Parasitologists talk of immunosuppression and virologists of immunodepression. The experimental evidence points to depression rather than suppression; the virologists' term is preferable and they used it first.

Interpreting heat flow

from our Geomagnetism Correspondent

IN the sense that heat plays a part, and very often a crucial part in all internal terrestrial phenomena, study of the Earth's thermal processes may be regarded as the most important of all branches of geophysics. But by the same token it is a difficult subject to come to terms with. Heat sources and sinks abound; yet data are sparse, difficult to determine and even more difficult to interpret. The very complexity of the situation militates against the drawing of significant conclusions; and as two new reports demonstrate, heat flow workers, through no fault of their own, can easily appear as the thermal counterparts of the Ancient Mariner who found "water, water everywhere, nor any drop to drink".

For example, Sass *et al.* (*Earth Planet. Sci. Lett.*, 21, 134; 1974) have taken advantage of geological investigations into possible sea level canal routes to measure heat flows in eastern Panama and north-western Colombia, the area where the Cocos, Nasca and Caribbean plates interact. There are, of course, few thermal situations more complex than the zones in which three lithospheric plates meet, for in such regions heat sources and sinks lie in close proximity to each other. A relatively cold lithospheric plate being thrust under an adjacent plate acts as a sink, but shearing along the boundaries between plates may produce frictional heating and thus a contribution to measured heat flow. Moreover, if in addition partial melting occurs, surface heat flow may be increased by the upward penetration of magma. These processes occur where two plates meet; at triple points they are even more intermingled.

In the present case Sass and his colleagues have obtained twelve new heat

flow values—three from the Canal Zone and three from each of three prospective sea level canal routes (routes 10, 17 and 25). In the vicinity of route 10, which lies 50–100 km west of the present Canal Zone, the average heat flow is high at $1.77 \mu\text{cal cm}^{-2}\text{s}^{-1}$ (h.f.u.). Previous work has shown that heat flow is also high south of Panama where it is associated with relatively young oceanic crust near the East Pacific Rise and the Galapagos Rift zone and with recent tectonic activity in the Panama Basin. The new land values, however, may be explained in terms of both the plate tectonic situation and the region's geological features. Sass *et al.* thus suggest that the high heat flow from route 10 may represent the easterly limit of a high heat flow zone associated with the Cretaceous volcanic centres which extend westwards into Costa Rica and Nicaragua.

Route 17, which lies 100–150 km south-east of the Canal Zone, and the Canal Zone itself are associated with heat flow values in the range 0.94–1.40 h.f.u. These are consistent with the low-to-normal values previously found at sea in the Caribbean plate to the north where, because of the oceanic character of the basement, it seems probable that most of the observed heat comes from mantle sources. The maximum contribution from the crust is thus probably no higher than 0.1–0.2 h.f.u. Some 100–150 km further south-east, along route 25, however, heat flow is even lower (0.66–0.70 h.f.u.). Because the average here (0.68 h.f.u.) is lower than even the theoretical 'mantle' heat flow found by Roy *et al.* (*In The Nature of the Solid Earth*, McGraw-Hill, 1972) for the Palaeozoic craton of North America, the observed surface heat flow presumably reflects the heat sink associated with the underthrusting Nasca plate.

In summary then, the new heat flow values obtained by Sass and his colleagues appear to be consistent generally with plate tectonics and surface geology even though they do little by themselves to sort out the complex interactions giving rise to them. By contrast, the analyses reported by Negi *et al.* (*Earth Planet. Sci. Lett.*, 21, 143; 1974) seem to be inconsistent even with previous work in the same field. Some years ago Horai and Nur (*J. geophys. Res.*, 75, 1985; 1970) carried out correlation and regression analyses of terrestrial heat flow (q) and thermal conductivity (k) which suggested that for many continental areas q and k are not independent. In fact, q correlated positively with k , a phenomenon which Horai and Nur put down to the fact that, in an inhomogeneous crust, heat flow will converge where the crust is more conductive. They further proposed that since q is proportional to heat production (A), k must also be proportional to A —a conclusion whose

necessity was later disputed by Naidu (*J. geophys. Res.*, **76**, 3426; 1971) but reaffirmed by Horai and Nur (*J. geophys. Res.*, **76**, 3428; 1971). A further conflict then came to light when it became clear that although Horai and Nur had found that the positive q - k correlation applied to the Indian peninsular shield, Gupta and Rao (*Bull. natn. Geophys. Res. Inst.*, **8**, 87; 1970) had already found no such correlation in the Indian Precambrian crystalline regions.

Continuing along similar lines, and to make matters even more confused, Negi and his coworkers have found that for all Indian sedimentary basins q correlates with k negatively. On the other hand, a positive correlation between heat flow and geothermal gradient (G) agrees with that found by Gupta and Rao for Indian Precambrian crystalline areas. Finally, heat flow is found to correlate negatively with crustal thickness.

What does it all mean? Negi *et al.* conclude that for sedimentary basins, at least, the negative correlation between q and k implies very little heat generation in the crust; in other words, most of the observed heat comes from below. The observed correlations between q , k , G and crustal thickness are often presumed to be due to variations in crustal thickness which produce a variable shielding effect on subcrustal heat. These are rather mundane conclusions to emerge from so much work; and it is difficult to judge their importance. Clearly, however, the parameters involved are very basic to the whole question of variations in heat flow; and so it would be helpful if someone would put all the data together and, if possible, explain just what is to be made of them.

Semiconductors in the human body?

from our
Solid State Physics Correspondent

It was realised about 20 years ago that the production of energy from oxygen in the cell mitochondrion was possibly the result of direct electronic transport through haemoproteins rather than the interaction of mobile ions through an aqueous medium (see, for example, Szent-Györgyi, *Discuss. Faraday Soc.*, **27**, 111; 1959; Carden and Eley, *ibid.*, 115). It was thus necessary to think about a sort of wet solid-state physics which could embrace reasonably high rates of transport of free electrons or holes through biological solids, especially through certain haemoproteins involved in respiration. Szent-Györgyi proposed a theory that a suitable conduction band could be produced by orbital overlaps in amino acid chains and one could predict from

this theory that proteins might achieve conductivities in the semiconductor range. The theory was conceptually very attractive but was not widely taken up, largely because it was not at the time susceptible of proof, nor had any investigation ever revealed a way of making analogous but simpler organic materials conduct to the level required.

Now, at least, one biological material has been shown to have a strikingly large conductivity when correctly excited. McGinness, Corry and Proctor, of the University of Texas Cancer Center, Houston, report in *Science* (**183**, 853; 1974) that melanins can be made to 'switch' from a poorly conducting to a highly conducting state at fairly low electric fields (say from 10^5 Ω cm to 10^3 Ω cm at a field of 3×10^3 V cm $^{-1}$). This remarkable phenomenon occurs both in melanin made synthetically from tyrosine and in that extracted from a human melanoma. The large conduction is not destructive in any way and is reversible; according to some tests, conduction seems to be electronic rather than ionic. Also tests of a few other likely biological materials in the same form (a compressed solid pellet inside a quartz tube, mildly hydrated and of length ranging from 0.1 to 10 mm) suggest that the effect is confined to the melanins and the authors note a similarity in the I - V characteristics of the sample to those of some amorphous inorganic semiconductors which undergo 'threshold switching'. But apart from the major difference in the electric field at which the threshold effect occurs (of the order of 10^3 V cm $^{-1}$ for melanin and 10^5 V cm $^{-1}$ for chalcogenide glasses), the current theory of the inorganic switching phenomenon rests on filamentary conduction, leading to a controlled degree of segregation of the constituents of the glass (for example, segregation of pure tellurium from Ge-Te alloys) and possibly strong injection at the electrodes under the high local fields (Bosell and Thomas, *Phil. Mag.*, **27**, 665-81; 1973).

Neither of these effects seems even likely in the system described, especially since the switching becomes unstable at thicknesses of chalcogenide greater than a few micrometres. Thus, the suggestion of McGinness *et al.* that melanin in the human body can be a cause rather than a by-product of disease and that its mode of action can be related to this 'electronic device' action is probably premature, especially considering the preliminary nature of the experiments. A revival of discussion on *in vivo* electronic effects in some biological materials associated with oxidation-reduction is, however, welcome if only because science has perhaps moved far enough since the 1950s that it can now devise adequate tests for the basic

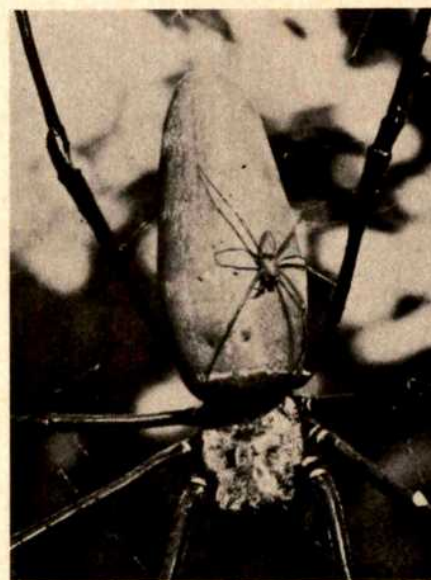
theories of transport in wet solids. Also a new approach to the treatment of melanotic diseases may well be stimulated by this particular revival of an intellectually stimulating discussion.

How to wrap up and get your female

from our
Animal Behaviour Correspondent

MICHAEL and Barbara Robinson spent a year in New Guinea observing the giant wood spider, *Nephila maculata* (Fabricius). Their report (*Smithson. Contrib. Zool.* No. 149; 1973) contains some remarkable facts of spider life, including a description of the strange method of courtship. As can be seen from the photograph, the male is much smaller than the female (4-6 mm body length as opposed to 40-50 mm) and before actual copulation, he goes through an elaborate 'lashing' of the female, weaving silk onto her body. The male may spend the greater part of two days standing on the female and during this time periodically indulges in intense bursts of activity, laying down silk threads between the female's legs and body. The female rarely gives any response at all to this silk deposition, after which the male copulates.

It would seem logical to suppose that the functional significance of binding up the female in this way would be that it in some way restrains her and prevents her from attacking the male. But although a great deal of silk is laid down around the leg bases, this does not seem to hamper the female very much, for she can rush off on predatory excursions even after the silk



Male on dorsal surface of the abdomen of a female *Nephila maculata*. Silk lines are visible on the thorax of the female leading to the leg bases and abdomen.

has been heavily deposited. The Robinsons suggest that the silk may in fact prevent the female from bending at the waist, which would in turn prevent her from picking off the male while he is copulating.

As well as describing the unique courtship, the Robinsons also describe the kleptoparasites of *Nephila*, spiders of other species which live in the web and steal food caught by the host. Often, the kleptoparasites were seen to rush in and start feeding while *Nephila* was actually subduing or wrapping its prey, a somewhat hazardous past-time as the kleptoparasites themselves often became enmeshed.

Origin of antibody diversity

from a Correspondent

THE apparently inexhaustible repertoire of antibody-combining sites possessed by vertebrate animals raises an important question. Does the germ line genome code for all antibody genes (germ line theory), or is diversity generated during the life of an individual by mutation of a restricted number of germ line genes (somatic theory)? Argument surrounding this question has raged backwards and forwards ever since the discovery that heavy and light chains of immunoglobulin molecules are composed of constant (C) and variable (V) regions (Hiltschmann and Craig, *Proc. natn. Acad. Sci. U.S.A.*, **53**, 1403; 1965). What is agreed is that separate genes code for C and V regions and that there is only a single C gene for a given constant region (Ig class or subclass) sequence. The question may therefore be reframed in terms of V genes: are all the conceivable V genes carried in the genome or are they somatically generated?

Perhaps the strongest argument against the germ line theory is the existence of allelic markers and species-specific amino acid sequences in V regions. But this argument is inferential and it has long been the hope that direct experimental evidence could be brought to bear on the question. This now seems to be provided by Premkumar, Shoyab and Williamson (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 99; 1974). In principle, the authors have isolated heavy chain messenger RNA and have estimated by hybridisation the number of C and V genes in isolated DNA. Their results, they argue, firmly support the germ line theory.

An absolute prerequisite for this type of experiment is that the isolated messenger RNA is pure and labelled at high specific activity so that hybridisation can be done in great excess of DNA. In order to achieve this, cells

from mouse myeloma MOPC 315 were labelled *in vitro* with radioactive uridine and cytidine, and then mRNA for the α chain was isolated from the poly(A)-containing fraction taking advantage of its specific interaction with immunoglobulin (Stevens and Williamson, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1127; 1973; *J. molec. Biol.*, **78**, 517; 1973). The resulting RNA sedimented largely as a single peak at 16-17S and, after sonication to 6-7S, was hybridised with about a 10^7 excess of cold DNA, also sonicated to 6.4S.

Using whole mouse embryo (the actual age is not given) as a source of DNA, a biphasic C_{ot} curve resulted. Approximately 70% of the input RNA was hybridised at the maximum, and 20% was found in the low C_{ot} transition. It is here that the authors make a crucial assumption, namely that the low C_{ot} transition (C_{ot} of 1.5) corresponds to hybridisation of V genes and the high C_{ot} transition (C_{ot} of 10^3) represents hybridisation to C genes. If this assumption is wrong, then so are the conclusions. Their reasons for making this assumption are that the RNA is pure, and that the ratio of hybridisation found in the two regions of the C_{ot} curve (0.29) is close to the expected figure of 0.25. The possible occurrence in the isolated RNA of RNA sequence stretches which do not code for C or V region sequences is obviously a source of uncertainty.

Similar, but not quite as impressive, results were obtained when BALB/C spleen or myeloma MOPC 315 DNA was used for hybridisation. Given the basic assumption made, the C_{ot} values correspond to 4-8 C genes and about 5,000 V genes.

Although sequence analysis indicates considerable cross-homology of mouse V_H regions, the figure of 5,000 V_H genes if accepted, must be a lower estimate. If, as the authors assume, that there are an equal number of V_L genes and that V_L and V_H regions can associate at random, then there is sufficient information for at least 2.5×10^7 antibody-combining sites. Even allowing for inefficiency, this would seem to be a high enough figure to account for antibody diversity. The authors conclude that their data "obviates the necessity to search for somatic generators of diversity. Questions concerning the maintenance and control of a large V-gene pool in the germ line and the expression of V genes by interaction with C genes can now be brought more clearly into focus".

Until the fraction of RNA hybridising at the low C_{ot} transition is actually identified as specifying V region, however, proponents of the somatic theory are unlikely to be satisfied and the argument will continue with unabated force.

Sense of supercoils in closed circular DNA

from a Correspondent

AN interesting study on the sedimentation of denatured closed circular DNAs with different degrees of strand interwinding has been made by Schmir, Révet and Vinograd (*J. molec. Biol.*, **82**, 34-35; 1974). From their results these authors have deduced the absolute sense of the supercoils in closed circular DNA and are able to confirm that the binding of intercalating dyes unwinds, rather than winds up, the DNA helix.

Double stranded DNA in the form of closed circular superhelices was first discovered in extracts from polyoma virus in 1963, and has subsequently been shown to exist in a wide variety of organisms. The biological significance of the closure is not yet clear but these molecules have a number of interesting structural and chemical properties. The superhelical structure imposes upon the DNA molecule a topological restraint usually expressed by saying that, for a given molecule, the total number of rotations of one strand about the other, the topological winding number, is invariant. Thus a modification in the number of turns in the basic double helix, for example by changing the ionic strength of pH of the environment, or by the addition of intercalating dyes, is accompanied by an equal and opposite change in the number of superhelical turns. The introduction of a single scission into one of the DNA strands results in a circular DNA with no superhelical structure. The superhelical DNAs sediment at a higher rate than the nicked DNAs, the exact value of the sedimentation coefficient, s , being a complex function of the superhelix density, σ (the number of superhelical turns per ten base pairs).

Vinograd, Lebowitz, Radloff, Watson and Laipis (*Proc. natn. Acad. Sci. U.S.A.*, **53**, 1104; 1965) studied the change in s for a DNA in which the topological winding density, $A \approx 1$, as the pH of the environment is progressively increased and the DNA becomes more denatured. At pH ≈ 12.5 , the DNA duplex is completely denatured, extensive positive supercoiling occurs and the sedimentation coefficient in these conditions is 2.1 times greater than that of a single stranded, circular molecule having an equivalent molecular weight. This greatly increased sedimentation coefficient can therefore be attributed directly to the winding of one strand about the other.

In the new article, Schmir *et al.* extend these results by studying the sedimentation coefficients, at high pH values, of DNAs with different topological winding densities. A number of closed circular DNAs with A values

varying from 0.99 to 0.89 were prepared from simian virus (SV40) and phage PM2 DNAs. This 10% decrease in topological winding density results in a decrease of approximately 5% in the alkaline sedimentation coefficient, which can be understood in terms of a small negative change in the superhelix density of the denatured molecule. The data connect smoothly with similar results obtained by Sebring, Kelly, Thoren and Salzman (*J. Virol.*, **8**, 478; 1971) for DNAs with A values from 0.6 to 0.15. The range is completed by the inclusion of a point for $A \approx 0$ computed from a relation proposed by Wang (*Biopolymers*, **9**, 489; 1970). As A decreases from 1 to 0, the value of relative s changes from 1 to 0.45.

The A values quoted here were determined on the assumption that for all naturally occurring closed circular DNAs, $A \leq 1$. This arises from the interpretation of the experimental results obtained from studies on the binding of intercalating dyes to superhelical DNA in accordance with the proposal by Lerman (*J. cell. comp. Physiol.*, **64**, suppl. 1, 1; 1964) and Fuller and Waring (*Ber. Bunsenges. phys. Chem.*, **86**, 805; 1964) that intercalating dyes unwind the DNA helix. The observed data are then consistent with a model in which the superhelix density σ is initially less than 0 and A is not greater than 1.

The recent assertion by Paoletti and LePecq (*J. molec. Biol.*, **59**, 43; 1971) that ethidium bromide winds up the DNA helix has the corollary that $A \geq 1$ in naturally occurring closed circular DNAs. Schmir *et al.* are able to refute this suggestion completely. First, they point out that the data of Sebring *et al.* were derived from replicating intermediates of SV40 DNA which were extensively unwound while nicked during the replicating process. There can therefore be no doubt that for these DNAs, $A \ll 1$. In addition, the point computed by Wang is unaffected by considerations of winding or unwinding the helix. Second, they have carried out dye titrations with a closed circular replicative mitochondrial DNA before and after removal of a short progeny strand (Révet, Schmir and Vinograd, *Nature new biol.*, **229**, 10; 1971). These experiments indicated quite clearly that less ethidium bromide was required to relax the supercoiled DNA molecule in the presence of the progeny strand than was required after its removal. The removal was achieved in such a way that the parent DNA did not become denatured and was able to rewind into its original structure. The results of Schmir *et al.* indicate unequivocally that the insertion of a displacing progeny strand and the binding of ethidium bromide act in the same direction, that is, they both unwind the DNA helix.

Schmir *et al.* conclude therefore that the initial superhelix density must be negative, A is less than 1 and the sense of the supercoils in natural closed circular DNA is negative, not positive as proposed by Paoletti and LePecq.

The results obtained during the past ten years on the binding of certain dyes to superhelical DNAs can be interpreted almost without exception in terms of a model in which the bound dye produces an unwinding in the DNA double helix. In view of the extreme sensitivity of superhelical DNA to small changes in its structure, this technique might in the future provide a means of distinguishing between the different modes of interaction of DNA and a variety of dye molecules.

Torpor in an Andean hummingbird

from our Animal Ecology Correspondent

THE attraction of hummingbirds to physiologically-inclined ecologists is easy to understand. How do these tiny, active creatures manage to survive in the inhospitable habitats of some of the world's highest mountains? Calder and Booser (*Science*, **180**, 751; 1973) found that nocturnal torpor during incubation was not uncommon at times of energy depletion. When the ambient temperature was low and heavy rain precluded nocturnal foraging, a gradual lowering of the body temperature ensured the sufficiency of the bird's energy reserves to see it through the night.

Another physiological adaptation of obvious survival value has been reported by Carpenter (*Science*, **183**, 545; 1974) who worked with a population of the hillstar hummingbird *Oreotrochilus estella*, which lives at a height of between 3,800 m and 4,300 m in the high Andes. By day the birds forage for nectar from a variety of local and exotic plants. At night they roost communally in caves and can readily be observed with little disturbance. Carpenter recorded body temperatures from a small group of birds: most were recorded between one and eight times during either the summer or winter study periods; a few were recorded in both summer and winter. Torpor was defined as a significant drop from the normal body temperature of about 30° C. In summer the average duration of nocturnal torpor was 7 h and during the winter 10 h. This difference is significant in spite of the fact that winter nights were 15 to 90 min longer than summer nights. Calder and Booser reported that body temperature during nesting torpor never sank below 6.5° C. The same was true in Carpenter's study, even though ambient temperature sometimes dropped to 3° C.

Why is torpor used more extensively



Zoological Society of London

Stripe breasted starthroated hummingbird (*Heliomaster squamosus*), a different species of hummingbird from that described by Carpenter but it shows the characteristic feeding behaviour of these birds.

by the birds in winter? One of the winter groups of birds lives in an area surrounded by blooming *Eucalyptus* trees, from which they fed heavily. Incidence and duration of torpor was the same as in other local populations with less well endowed larders. It seems as if energy depletion is not a cue for torpor. Nor does temperature seem to be the trigger. In summer the ambient temperatures in the roosts varied from 3.5° C to 13° C, and in winter from 3.0° C to 13° C. This high winter temperature is thought to be exceptional; winter roost temperatures are normally near freezing.

Carpenter suggests that although the difference between summer and winter ray length is brief, it is sufficient to act as a photoperiodic cue for a circannian rhythm. Such a rhythm would have survival value for a species living in an area where nocturnal temperature is seasonally low and in which the day time temperature gives no indication of the ensuing night time level. By entering torpor as soon as it roosts, a bird is capable of regulating its body temperature for long periods should the ambient temperature drop much below 6.5° C—a clever way of extending fuel contingency reserves without increasing fuel capacity.

Probing the secrets of ionic channels

from a Correspondent

SINCE Galvani's fortuitous joining of brass hook, iron plate and frog in 1786, the lure and mystery of 'animal electricity' have been reduced to the molecular secrets of so-called cation channels, which intermittently allow these ions to cross membranes. Recent chemical and electrical probes of the structure of these channels were the main topics of a meeting at the Royal Society on March 13-14.

The simplest model of a channel is a pore, a few Angstroms in diameter, capped by one or more gates which open or close the pore depending on the recent history of the voltage across

the membrane. B. Hille (University of Washington) summarised the evidence for such models for the sodium and potassium channels, as gleaned from the voltage clamp technique pioneered by Hodgkin and Huxley more than 20 years ago. Hille's and other data (Woodhull, *J. gen. Physiol.*, **61**, 687; 1973) suggest the presence of a carboxylic acid group inside the sodium pore.

The same carboxylic acid group was detected by J. M. Ritchie (Yale University) and colleagues, using a powerful technique not requiring electrical measurements (*J. Physiol.*, **232**, 53P; 1973). They deduced its presence from the competitive binding of tritiated tetrodotoxin (TTX) and other ions to the sodium channel. TTX binding survives solubilisation of membranes in Triton X-100, but the sodium channels have yet to be purified by this route. By contrast, the much tighter binding of α -bungarotoxin to acetylcholine receptors has aided their purification, as discussed by D. P. Green (University College, London).

The lack of a specific toxin for the potassium channel was widely lamented, though vagaries of the potassium currents in nodes of Ranvier and in cardiac muscle were described by B. Frankenhaeuser (Karolinska Institute, Stockholm) and D. Noble (University of Oxford). P. F. Baker (University of Cambridge) discussed delayed movements of calcium following action potentials, which he thinks are related to those activating transmitter release at synapses. L. G. M. Gordon and D. A. Haydon (University of Cambridge) talked about quantal permeability changes in artificial membranes, and G. K. Radda (University of Oxford) described the use of fluorescent probes.

A. von Muralt (Universität Bern) and J. V. Howarth (Marine Biological Association, Plymouth) summarised the slight optical and thermal changes in membranes during action potentials. These, it was concluded, reflect the consequences rather than the causes of the underlying permeability changes.

The latest and most controversial technique involves measuring the tiny displacement currents visible in voltage-clamped axons when the usual ionic currents are blocked. C. M. Armstrong and F. Bezanilla (University of Rochester) think that these are 'gating currents', caused by the movement of those charged particules or dipoles thought to open and close the sodium channel (*Nature*, **242**, 459; 1973), and they reported, as supporting evidence, that perfusion of an axon with $ZnCl_2$ removed reversibly both sodium and displacement currents. Both currents could also be inactivated by depolarising pulses, and this effect was removed by pronase.

H. Meves (MBA, Plymouth) took

issue with this interpretation, pointing out that other charged groups, which may vastly outnumber the gating groups, could easily obliterate the true gating current. Furthermore, although the qualitative behaviour of the measured displacement currents is acceptable, he observed that they depart from expectations in several quantitative respects. W. K. Chandler (Yale University), who first studied similar but slower currents in muscle (*Nature*, **242**, 244; 1973), suggested that they play a part in excitation-contraction coupling.

If gating currents are what their name implies, then their magnitudes may be used to calculate the density of sodium channels, if one assumes six net charges per channel as given by Hodgkin and Huxley. R. D. Keynes (University of Cambridge), who organised the conference, and E. Rojas (MBA, Plymouth) estimated on the basis of both displacement currents (*J. Physiol.*, **233**, 28P; 1973), and TTX binding, that the squid giant axon has about 400 sodium channel per μm^2 . A. L. Hodgkin (University of Cambridge), using a dimensional argument, suggested that the density of sodium channels in nerve should be about 1,000 per μm^2 , if the conduction velocity is to be maximised. These numbers are much higher than the 13 channels per μm^2 originally estimated on the basis of TTX binding to lobster nerve (Moore, Narahashi and Shaw, *J. Physiol.*, **188**, 99; 1967) and popularly subscribed to until about the middle of this meeting. The discrepancy suggests either vast species differences, or errors in the interpretation of TTX binding studies (tritiated impurities were mooted), or that 'gating' currents contain a large component that has nothing to do with gating. *Caveat emptor.*

Cloning for obesity

from our
Molecular Genetics Correspondent

NATURAL selection of organisms for traits desirable under particular circumstances is of course a well established feature of evolution. And artificial selection has been used both to isolate mutants of bacteria or eukaryotic cells in culture and to permit only cells possessing certain functions to survive. Cloning cells in culture can be used to isolate lines with enhanced or reduced expression of functions which can be followed quantitatively (rather than the all or nothing expression of many mutants). In a classic series of experiments some years ago, the 3T3 and 3T6 cell lines of mouse fibroblasts were derived with different responses to inhibition of growth by culture conditions. By cloning sublines of the 3T3 cell, Green and Kehinde now report in

the March issue of *Cell* (**1**, 113; 1974) that they have isolated two sublines which accumulate large amounts of triglyceride fat in the resting state.

Illustrating their article with some striking colour photographs, Green and Kehinde demonstrate vividly that the cells of these sublines resemble adipose cells in accumulating large amounts of lipid in their cytoplasm. The initial observation which led to these experiments was the presence of a few cells possessing liquid droplets in resting cultures of 3T3 cells; when resting cells are transferred to conditions which promote growth, the fatty cells are not found among the growing progeny, although such cells reappear when the culture again enters the resting state. By isolating areas rich in lipids from resting cultures, growing these isolates and then repeating the process when the culture reached the next resting phase, Green and Kehinde obtained cultures which were progressively richer in fatty cells. Eventually, they isolated two clones which accumulate lipid in large amounts.

When resting cultures of these cells are transferred to conditions which stimulate growth, the most fatty cells may survive for a few days but usually disintegrate eventually. The presence of large amounts of fat presumably interferes with the processes of cell division. But cells possessing less fat may reinitiate division; and after a successful division — sometimes mitosis fails in cytokinesis — the amount of lipid is diluted and then continues to decrease in each cell during successive cycles; except for the extremely fatty cells, therefore, both the lipid-accumulating sublines can be propagated indefinitely.

What is the cause of the lipid accumulation? Possible explanations, at present unresolved, include excessive uptake from the culture medium, increased synthesis within the cell or decreased degradation. Because the accumulation of lipid is inhibited by lipolytic agents such as dibutyryl cyclic AMP, Green and Kehinde suggest that one practical use of these lines may be to provide a means to test drugs for possible effects on lipid metabolism. And even more intriguing are some of the possible theoretical implications of the properties of these cells. What genetic change may underlie the conversion of a cell derived from the fibroblast into one apparently akin to the cells of adipose tissue? How is this change related to the usual processes of differentiation in developing mouse cells and can such changes be found for other phenotypes? The development of a differentiated phenotype in culture represents an important contrast with the more usual consequence of tissue culture.

Spatial resolution of ERTS images by computer

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The authors discuss a solution to one of the problems concerned with the use of the large amounts of data now available from remote sensing satellites.

A PERSISTENT problem in the remote sensing of Earth resources is the difficulty of gaining efficient access to the vast quantities of data which have been generated. Second only to this problem is that of displaying the results in a form convenient for analysis. Ever since the advent of remote sensing satellites, one of the aims of imagery analysis has been to modify the data in order to delineate and enhance specific features within a composite display. The aim of many investigators of data handling is to develop a man-computer integrated system for quickly and accurately enhancing and interpreting a large volume of data from an Earth resources satellite. Since the early 1960s, there has been extensive research on the development of spectral signature and pattern recognition theory in processing multispectral data. The thrust of this research has been to develop computer software packages to analyse large amounts of data generated by multispectral scanning instruments. It has been only recently, however, that image-display man-computer hardware systems have been developed.

Feasibility

The University of Wisconsin's Space Science and Engineering Center (SSEC) has demonstrated the feasibility of the Man-computer Interactive Data Access System (McIDAS) which has rapid data storage, access, display and analysis techniques applicable to many areas of satellite data processing. In this field, it is of considerable value to reference small subsets of these data in a fast and economical way, with accompanying observation and guidance by a human operator who interacts with the system. The advantage of the McIDAS system in the analysis of ERTS data is that the operator can see the data displayed on a television screen in a form suitable for easy interpretation by varying such quantities as the brightness and colour enhancement functions, time lapse motion and scale sizes. He can make the appropriate judgments, then communicate exact coordinates and instructions for processing of the digital data. In fact, the system always has access to the full precision data, so that maximum accuracy is always possible.

We are developing the software programs specifically for this system to analyse remote sensing data from satellites for natural resource inventory purposes and for monitoring changes in land use. Normally, investigators of Earth resources use aerial photographs to obtain inventory estimates of the required accuracy but not without expending time in the preparation of photographs, tedious interpretation of them and data handling. Lins and Milazzo¹ have described the use of multispectral photography for detecting land-use change. Wray² has made a comparative study of metropolitan regions using ERTS imagery; he states that the imagery shows great promise for the inventory of gross land use and the monitoring of changes in land use³.

The purpose of this paper is to present results from McIDAS showing the spatial resolution that can be obtained from ERTS-1 digital data by the display of urban images appropriate for identification of land use.

Four reels of ERTS-1 system corrected computer compatible tapes from the multispectral scanner (MSS), containing a scene of eastern Wisconsin taken on September 14, 1972, was made available to us by NASA. After modifying the data format for the McIDAS system, the Milwaukee metropolitan area was displayed in all four channels sequentially. A number of images were then manually enhanced by means of a teletype keyboard in which various levels of brightness values of the raw ERTS data were transformed to a chosen level of brightness within the dynamic range of the Muirhead PhotoFax Receiver. In the case of the pictures shown here, this involved 128 brightness levels from the MSS 5 channel transformed nonlinearly into 256 levels for the Muirhead Receiver. By using a number of trial and error non-linear enhancement curves to display the images on the McIDAS CRT, we were able quickly and economically to find the enhancement curve suited to the display of urban imagery in such a way that a number of recognisable features can be seen.

In Fig. 1, the major highways and roads leading to Milwaukee can be easily identified. The downtown area is located near the centre of the picture where East-West Interstate 94 turns South. An effluent plume into Lake Michigan can be seen immediately to the south-east of the downtown area just beyond the place where the Menomonee and Milwaukee Rivers merge. Other land features can also be seen, such as the Milwaukee County Stadium and the Forest Home Cemetery.

The area shown in Fig. 2 is one-fourth that encompassed by Fig. 1. In Fig. 2, the individual data points which make up the imagery become somewhat discernible. The plume at the estuary of the rivers, as well as the variation in reflected radiance from Lake Michigan near the shoreline east of Mitchell Field, is more apparent than in Fig. 1.

In Fig. 3, the individual ERTS data points are now quite evident. Mitchell Field appears here as a pattern of blocks.



Fig. 1 ERTS-1 Mufax picture from McIDAS digital computer enhancement of Milwaukee, Wisconsin. MSS 5 red visible channel taken on September 14, 1972. The picture comprises 360 ERTS scan lines (27.4 km) and 540 ERTS elements (30.8 km). a, Milwaukee river; b, Lake Michigan; c, Menomonee river; d, stadium; e, plume; f, highway 94; g, cemetery; h, Mitchell Field.



FIG. 2 ERTS Mufax picture from McIDAS digital computer enhancement of central and south Milwaukee. MSS 5 red visible channel taken on September 14, 1972. The picture comprises 180 ERTS scan lines (13.7 km) and 270 ERTS elements (15.4 km). *a*, Pier; *b*, plume; *c*, highway 94; *d*, Lake Michigan; *e*, cemetery; *f*, golf course; *g*, Mitchell Field.

The limits to the ability of ERTS to resolve features is clear; any further enlargements would not improve spatial resolution for the viewer. Other enhancement curves would, however, help delineate particular features not considered here.

Our software programs developed so far allow for three sizes of image field which represent the magnification ratios of 1:2:4, designated by the last digit on the annotation block in the three figures as 3, 2 and 1 respectively. The following information concerns Fig. 3. Information corresponding to Figs 1 and 2 can be obtained simply by using the scale factors 4 and 2, respectively.

In Fig. 3 the total image field represents 90 ERTS scan lines with 135 ERTS samples comprising each line. The 90 lines \times 135 samples represents a physical field size of approximately 6.8 km \times 7.7 km, respectively. Because of the size of the individual data points represented in the scanning display, each ERTS element had to be repeated twice to ensure that the proportions of the imaged field would correspond closely to the actual geographic proportions. The scaling, however, is still not quite exact. In the figures, the vertical dimension of the image is 'stretched' by 6.8% with respect to



FIG. 3 ERTS-1 Mufax picture from McIDAS digital computer enhancement of South Milwaukee. MSS 5 red visible channel taken on September 14, 1972. The picture comprises 90 ERTS scan lines (6.8 km) and 135 ERTS elements (7.7 km). *a*, Lake Michigan; *b*, golf course; *c*, Mitchell Field.

the horizontal dimension. The enhancement curve for each of the figures shown was:

Raw			
ERTS	0 to 7	\rightarrow 0	} Enhanced display
digital	8 to 64	\rightarrow 0 to 255 (linear)	
data	65 to 128	\rightarrow 255	

We thank Mr Ralph DeDecker for developing the software programs, Professor James Clapp for use of the ERTS digital tapes and Professor Verner E. Suomi and Mr Thomas O. Haig for discussions.

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Mesozoic thermal events in southeast China

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The first Rb-Sr age data are reported for Mesozoic batholiths in southeast China. Two thermal episodes are recognised (165 ± 13 and 90–109 Myr) and can be correlated with the periods of rapid spreading of the Mesozoic Pacific Ocean floor as proposed by Larsen and Pitman.

FROM the study of Mesozoic magnetic lineations in the western Pacific, Larsen and Pitman¹ and Larsen and Chase² postulate that, during the late Mesozoic, the Pacific sea

floor was spreading from at least five spreading centres joined at two triple points. Most of the Pacific Basin today is occupied by the expanded Pacific plate of the late Mesozoic system. This implies that an area equal to most of the Pacific Basin has been subducted beneath the surrounding continents since the early Cretaceous. Larsen and coworkers also correlate the Keathley magnetic lineations of the North Atlantic with the Hawaiian lineations of the Pacific and suggest that the Bay of Biscay opened during the interval between 150 and 110 Myr BP and the drift in the South Atlantic was initiated at sometime during the interval from 110 to 85 Myr BP. More importantly, with their magnetic

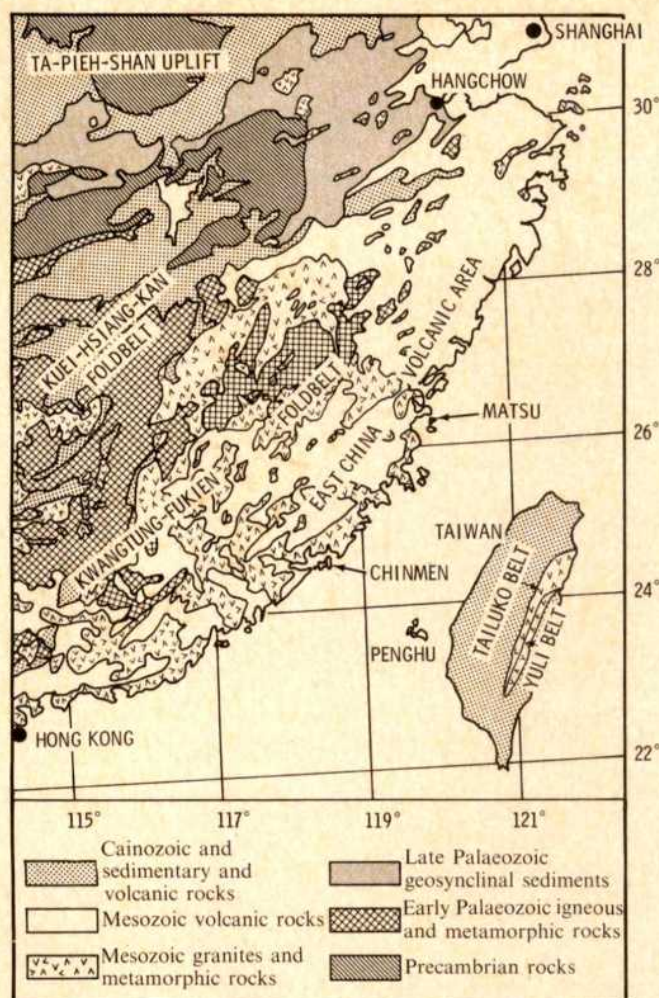


FIG. 1 Generalised geological map of southeastern China and Taiwan. Source, refs 11 and 28.

reversal time scale, they show that from 110 to 85 Myr there was a pulse of very rapid spreading in the central and south Atlantic and throughout the Pacific (up to 18 cm yr⁻¹ along the Phoenix-Pacific spreading centre).

With a rigid plate assumption, rapid spreading corresponds to rapid subduction and consumption of oceanic plate materials beneath the surrounding continents. (This statement is not strictly true, for subduction may be beneath oceanic but not continental lithosphere, for example, in the western Pacific. However, from an analysis of tectonic activities along the Pacific margins during the Mesozoic time, this statement is generally true.) If andesitic or granitic magmas of continental margins and island arcs were derived from the under-thrust lithosphere or had any genetical relationship with the subduction, one may anticipate a correlation between spreading rate and the intensity of orogeny and associated thermal episodes (andesitic volcanism, batholithic intrusion and metamorphism). Indeed, Larsen and Pitman¹ related the pulse of rapid spreading from 110 to 85 Myr BP to episodes of circum-Pacific intrusive and extrusive activity and orogenic movement during this period.

Here, I report a new set of age data on some granitic batholiths of southeast China. I shall show that these new data support the postulate of Larsen and Pitman.

The geotectonic element of which the southeast China coastal region is a part was named 'Cathaysia' by Grabau³. It includes the South China Basin, Taiwan, the Yellow Sea and Japan, and it was suggested to be an 'old land' of Precambrian age. This 'old land' supplied detritus to a Cathaysian geosyncline to the northwest, only to disappear as the

geosyncline later migrated southeastward. But Hsieh⁴ rejected Grabau's concept of an 'old land' and suggested that the bulk of the Kuei-Hsiang-Kan and the Kwangtung-Fukien Foldbelts (Fig. 1) are younger Caledonian, and postulated a Hercynian foldbelt north of Canton (not shown in Fig. 1, but located about 50 km northwest of Hong Kong) and a larger Mesozoic foldbelt along the coastal region of southeast China.

The basement rocks of southeast China are principally metamorphic rocks of various facies associated with intrusive granites. Their ages are not known. Toward the coastal areas, volcanic rocks, also associated with intrusive rocks, comprise the widespread East China Volcanic Area. It has been speculated that they are of Jurassic-Cretaceous age. During the Mesozoic Yenshan Orogeny in China (whose early phase corresponding to the Nevadan Orogeny of the western United States) folding and faulting of sedimentary rocks were accompanied by volcanism and intrusion. Extensive Mesozoic granites were thus developed throughout the southeastern part of China. The period of Yenshan Orogeny includes the most important metallogenetic epochs of the whole of China⁵.

Dating the rocks

Rocks collected from two offshore islands (Chinmen and Matsu) were dated by the Rb-Sr method. All granitic rocks from Chinmen and Matsu, including granitic gneisses and biotite-hornblende gneisses, yielded mineral isochron ages of 93 to 109 Myr (Table 1). A late pegmatitic dike (K-4), which intruded a granitic body (K-3), was dated at 90 Myr. The initial ⁸⁷Sr/⁸⁶Sr ratios of these rocks vary with the Rb/Sr ratios of the individual rocks from 0.706 to 0.716. Dioritic and gabbroic rocks from Matsu yield similar ages (94.1 to 97.5 Myr), with a very narrow range of initial ratios of about 0.7065 to 0.70695. In addition, a primary age of 165 Myr, with an initial ⁸⁷Sr/⁸⁶Sr = 0.7055, was obtained from the whole rock data for granitic rocks, implying either that these rocks may be genetically related and of mantle origin with limited crustal contamination or that the original sedimentary sources for the granitic gneisses were Sr isotopically homogenised about 165 Myr ago.

The whole rock data for the mafic rocks from Matsu (diorites, gabbro and spessartite) are more scattered and

TABLE 1 Mineral and whole-rock isochron ages and initial ⁸⁷Sr/⁸⁶Sr ratios of some igneous rocks from Southeastern China

Sample No.	Locality	Rock type	Age (Myr)	(⁸⁷ Sr/ ⁸⁶ Sr) ₀
K-1	Chinmen	granite gneiss	95 ± 2*	0.7084 ± 1*
K-2	"	bio-hb gneiss	96.7	0.7060
K-3†	"	granite gneiss	109 ± 7	0.7079 ± 5
K-4‡	"	pegmatite	90 ± 3	0.7112 ± 1
K-5	"	granite gneiss	94.2 ± 3	0.7159 ± 5
K-6	"	granite gneiss	98.1 ± 12	0.7065 ± 7
K-7	"	bio-hb gneiss	—	0.7066‡
M-23	Matsu	diorite	97.2 ± 1.4	0.70693 ± 1
M-24	"	hb-porphyrite	—	0.7062‡
M-25	"	gabbro	97.5 ± 4.8	0.70695 ± 8
M-27	"	diorite	94.1 ± 0.3	0.70666 ± 3
M-31	"	spessartite	—	0.7065‡
M-06	"	granite	95.5 ± 4.0	0.70676 ± 8
MPK	"	granite	92.6 ± 2.0	0.70691 ± 3
Whole-rock§	Chinmen & Matsu	granites	165 ± 13	0.7055 ± 2

* Ages were calculated by the York method³⁰ using the decay constant of ⁸⁷Rb = 1.39 × 10⁻¹¹ yr⁻¹. Uncertainties for both ages and initial ⁸⁷Sr/⁸⁶Sr ratios are at 2σ level except for K-2 which is a two-point isochron. Uncertainties of initial ratios correspond to the last figures.

† K-3 was intruded by K-4 pegmatite dike.

‡ Initial ⁸⁷Sr/⁸⁶Sr ratios corrected for radiogenic ⁸⁷Sr growth in the assumed time interval of 95 Myr.

§ Whole-rock isochron was constructed using the data of K-1, K-2, K-3, K-5, K-7, M-06 and MPK.

TABLE 2 Mesozoic thermal events in circum-Pacific areas (based on radiometric dates)

Area*	Description and comment	References
Alaska-Aleutian Range	Three discrete episodes of intrusions are found: (1) early to middle Jurassic (176-154 Myr), (2) late Cretaceous and early Tertiary (83-58 Myr), (3) Middle Tertiary (38-26 Myr). All ages are K-Ar mineral dates.	14
Coast Range, British Columbia	Orogenic pulses took place from Proterozoic to late Tertiary. K-Ar dates suggest that plutonic emplacement and later uplift were most vigorous and widespread in Cretaceous and Tertiary.	15
Sierra Nevada	K-Ar and Rb-Sr data show that intrusion began in late Triassic (210 Myr) and ended in late Cretaceous (80 Myr). Emplacement of granites accomplished in five major epochs, regularly separated by 30 Myr intervals. Magmas were derived from the upper mantle or lower crust with some sialic contamination.	7, 16, 17
Franciscan Formation	K-Ar dates span from 150 to 70 Myr. Three major metamorphic events: (1) 150 Myr, (2) 90-110 Myr, (3) 70 Myr. These events are correlated with intrusive epochs in the batholith belt (example, Sierra Nevada).	9
SW United States	K-Ar dates indicate two broad patterns which culminate in (1) the Jurassic (160-130 Myr) and (2) the mid- to late Cretaceous (105-75 Myr). Essentially coeval with Franciscan and Batholith belt activities.	8
Baja California	U-Pb age = 96-118 Myr. Rb-Sr data indicate a mantle source for the Peninsular Range batholith.	18
Northern Chile	K-Ar biotite ages indicate four episodes of granitic intrusion: (1) lower Jurassic (182-190 Myr); (2) middle Cretaceous (87-107 Myr); (3) lower Palaeocene (60-67 Myr); (4) upper Eocene (40-43 Myr). Intrusive foci migrated eastwards.	19
Southern Chile	K-Ar and Rb-Sr ages indicate three major episodes of intrusion into the basement complex of Palaeozoic age: (1) 164-126 Myr; (2) 106-80 Myr; (3) 53-11 Myr. (Original Rb-Sr ages were recalculated using Rb decay constant = $1.39 \times 10^{-11} \text{ yr}^{-1}$).	20
Antarctica	Granites and granodiorites from the peninsular region of western Antarctica show a pattern of Mesozoic igneous activity similar to that of South Andes region. Rb-Sr ages clustered around 96-109 Myr.	21, 22
New Zealand	Radiometric dates for intrusive and metamorphic rocks on both sides of the Median Tectonic Line of the South Island cluster in the interval 95-120 Myr. The timing of the Cretaceous orogeny and paired metamorphism in New Zealand is similar to that in Japan.	23
Southeast China	Rb-Sr W. R. isochron age of 165 Myr suggests a primary thermal event for the Chinmen granites. Mineral isochron ages, which cluster in the interval 94-109 Myr, correspond to a secondary thermal event for the Chinmen granites and a primary intrusive event for the Matsu diorites and gabbros.	This paper
Taiwan	K-Ar age of 86 Myr for a muscovite from a quartz diorite which intruded agneissic rock in the Tailuko Belt (low P/T), immediately west of the Yuli Belt (high P/T type). The time of intrusion corresponds to that of the Nanao Orogeny in Taiwan and an episode of the Yenshan Orogeny in China.	12
SE Asia	Granites northeast of Gulf of Thailand are mainly Triassic or older. Widespread thermal events including granitic emplacement took place during Cretaceous to Tertiary. In Indonesia, granites from the Lassi Mass and the Lampong Mass of Sumatra and from offshore areas north of Java were dated at about 100 Myr.	24, 29
Japan	K-Ar dates indicate that most intrusion took place in early Cretaceous to early Tertiary (90%), and weakly continued to late Tertiary. Paired metamorphism occurred during middle Cretaceous time.	25, 26
South Korea	Rb-Sr data indicate that a thermal event (180 Myr) homogenised Sr isotopic compositions of mineral phases in a Precambrian rock. Whole rock data of five granitic rocks from the southeast coastal region yield an age of about 100 Myr.	27

* Areas arranged clockwise around the Pacific.

do not form any linear isochron relationship (details to be published by B. J., P. Y. Chen and T. P. Yen). Their initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.7065 to 0.70695, derived from mineral isochrons, suggest a possible mantle origin with some crustal contamination. It is quite certain, however, that the source materials for the Chinmen and Matsu granites were not solely Rb-rich crustal rocks of much older ages, such as the Precambrian strata of the Ta-Pieh-Shan Uplift.

In summary, the granitic rocks probably record a primary (165 Myr) and a secondary (about 100 Myr) thermal episode, while the unmetamorphosed diorites and gabbro from Matsu register only a time of intrusion also at about 100 Myr. The time interval of 90 to 109 Myr BP embraces an outstanding episode of the Mesozoic Yenshan Orogeny in China.

It is of great interest to see how the rapid seafloor spreading during the time interval of 110-85 Myr BP correlates with supposedly consequential dynamic events on the continents, especially in the circum-Pacific areas. Gilluly⁶ stated "The Middle Cretaceous was the time of emplacement of by far the greatest plutons of Phanerozoic time in the whole

belt extending from Alaska to Baja California. The batholiths of the Peninsular Range, the Sierra Nevada, many of the Coast Range plutons of California, the Idaho Batholith and many of its satellites in eastern Oregon and northern Idaho all belong to this episode. The plutons of northeastern Washington are outliers of the great Coast Range batholith of British Columbia." To supplement the evidence cited by Larsen and Pitman, I have collected some more supporting evidence shown in Table 2. Not all the age data in Table 2 explicitly indicates a strong peak during the 85 to 110 Myr interval in the age distribution pattern. This is easily understood if factors such as sampling bias and rates of uplift and subsequent erosion which exposed the intrusive rocks are considered. Furthermore, even during a period of rapid spreading, the rates of lithosphere consumption and accompanying igneous activities will not necessarily be increased uniformly around the Pacific margins. The possibility of a fortuitous coincidence between the fast spreading indicated by the magnetic anomaly time scale and the magmatic episodes should not be overlooked. Perhaps Larsen and Pitman's time scale is premature and may be changed after

more detailed study. Similar doubts about such a correlation and about the regular periodic magmatic events proposed by Evernden and Kistler⁷ for the Sierra Nevada were raised by Armstrong and Suppe⁸. Nevertheless, the present data for China are consistent with the interpretation of Larsen and Pitman.

Metamorphism with intrusion

Finally, I would like to call attention to the coeval occurrence of paired metamorphism and intrusion on both sides of the Pacific during the Mesozoic. On the east side the Franciscan terrain is parallel to and west of the batholith belt of California. The metamorphism of the Franciscan Formation occurred from 150 to 70 Myr BP, a time span which is essentially contemporary with the times of most extensive magmatism in the batholith belt⁹.

On the west side of the Pacific, there are the classical examples of paired metamorphic belts in Japan¹⁰, and the Yuli Belt of high pressure/low temperature glaucophane schist in Taiwan, about 170 miles east of Chinmen and Matsu (Fig. 1). Since no systematic radiometric dating has been done on these metamorphic rocks, a direct correlation is not possible. Based on field relationships, however, the metamorphism was thought to occur in late Mesozoic¹¹. Moreover, as shown in Table 2 a single K-Ar date of 86 Myr¹² was reported for muscovite from a quartz diorite which intruded a gneissic rock of the Tailuko Belt, with which the Yuli Belt is in fault contact. Thus the high-pressure type of metamorphism in Taiwan may be coeval and tectonically paired with the low-pressure type of metamorphism and plutonic intrusion in southeast China. This mirrors the situation in California. Yen¹¹ proposed that the Tailuko (low P/T type) and the Yuli belts (high P/T type) in eastern Taiwan (Fig. 1) formed the paired metamorphic belts which are now in direct fault contact. If the so-called arc-trench gap¹³ was ever present between these two metamorphic belts, it would require a great amount of crustal displacement to bring these two together. Perhaps a more logical way is to pair the Yuli belt with the batholiths of southeastern China. This specific problem will be discussed elsewhere (B. J., P. Y. Chen, and T. P. Yen, to be published).

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Free precession of neutron stars

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Wobble in pulsars can provide a clue to the structure and properties of the associated neutron stars.

DETECTION of free precession represents one of the most promising methods of determining both the mass and internal structure of a neutron star. The free precession frequency differs by some three (or more) orders of magnitude, depending on whether the star possesses a solid or a liquid core¹. Thus if one attributes the 35 d period of the high-low X-ray cycle observed for the compact X-ray source Her X-1 (ref. 2) to free precession of the neutron star Her X-1 (refs 3, 4) then from the magnitude of that period alone one can conclude that Her X-1 possesses a solid inner core

(of hyperons or neutrons)⁴. Here we examine ways of exciting and maintaining such precession, as well as mechanisms which act to damp it. This problem has been considered earlier by Goldreich⁵ and by Henriksen *et al.*⁶, whose starting assumptions and conclusions differ somewhat from ours. We conclude that crust-liquid coupling is probably the principal source of wobble damping and that for pulsars wobble excitation is more easily accomplished in a comparatively young, rapidly rotating neutron star. For the Crab and Vela pulsars we find that wobble damping is comparatively ineffective, so that a moderately effective pumping mechanism suffices to excite stellar wobble to an amplitude ($\sim 6^\circ$ to 15°) limited only by the maximum storage of angular elastic energy in the solid crust (or core) of the star.

Analogy with terrestrial wobble

To put the problem of exciting stellar wobble in a terrestrial perspective, we note first that 80 years after the discovery of the free precession of the Earth by Chandler in 1891, the mechanism which is responsible for exciting this Chandler wobble remains in doubt. It is now generally believed that the dissipation in shallow oceans of energy associated with polar tidal motions is sufficiently great that, left to its own devices, the Chandler wobble would damp in less than 100 yr (ref. 7). Yet after almost 100 yr of observation, the amplitude of the wobble (~ 6 m or 10^{-6} rad) remains essentially unchanged; a mechanism to pump the Chandler wobble is clearly required.

Three of the various suggested terrestrial pumping mechanisms are of particular interest to the astrophysicist interested in pumping the wobble of a neutron star: (1) random pumping by earthquakes^{8,9}; (2) random pumping by the core-mantle interaction¹⁰; (3) resonant pumping by earthquakes as a result of in-phase release of angular elastic energy, triggered by the Chandler wobble itself¹¹.

The first mechanism is too inefficient to maintain the Chandler wobble at its present amplitude¹²; its analogue may, however, be operative for a comparatively young neutron star with a solid core (the Vela pulsar?). To the extent that the interpretation of the giant Vela spin ups as produced by corequakes¹³ is correct, such corequakes could excite a substantial stellar wobble through the discontinuous jumps in the centre of nutation they bring about.

An analogue of the second mechanism—random angular momentum transfers between the core and crust of a neutron star—could well act to maintain the precession of a neutron star with a liquid interior (the Crab pulsar?). We lack, however, any physical picture for what might be causing such transfers (which would be reflected as frequency jumps analogous to the changes in the length of the day observed for the Earth); moreover, because of its randomness, such a mechanism is comparatively inefficient, so that pumping a large amplitude stellar wobble in this fashion may be difficult.

An analogue of the resonant pumping mechanism might operate in a neutron star like Her X-1, for which it has been suggested⁴ that the free precessional motion of the star acts to regulate the accretion of matter to the stellar surface. If corequakes in the solid core of Her X-1 are triggered by such accretion (and crustquakes could provide the catalyst needed to bring this about), we would then have a quite efficient self-regulating system: on the one hand, stellar wobble regulates the accretion of matter to the stellar surface (which we see on Earth as a high X-ray state); on the other hand, corequakes triggered by that accretion act to maintain the core wobble. Although the direct detection of such corequakes would be difficult (even in the case of a frequency jump, $\Delta\Omega/\Omega$, as large as 10^{-6}) such quakes could be indirectly detected through the changes in amplitudes of the stellar wobble they produce and may indeed have already been detected. A sizeable jump in the amplitude of the stellar wobble would alter the pattern of the high-low X-ray states, so that one would observe the high X-ray state for a greater (or smaller) number of days within the ~ 35 d overall cycle. Since such shifts in the high-low X-ray cycle have been frequently seen, it is tempting to attribute them to shifts in the amplitude of the stellar wobble; more work is, however, needed before such an interpretation can be taken seriously.

Pulsar mechanism

In a pulsar there is an additional mechanism which might be responsible for exciting stellar wobble—the radiation torque associated with the pulsar magnetic field. Goldreich⁴

has examined the change in nutation amplitude brought about by the electromagnetic torque associated with magnetic dipole radiation; he finds that free nutation grows or damps on the slowing down time scale ($T \sim 2,500$ yr for the Crab pulsar) depending on whether the angle, χ , between the magnetic axis and elastic reference axis exceeds or is less than $\sim 55^\circ$; the rate of growth is, however, proportional to the amplitude to nutation, so that it seems difficult to achieve an appreciable nutation amplitude in times which are not long compared to T . We have examined the problem from a somewhat different point of view; in considering the influence of a torque which acts perpendicular to the reference axis, n_0 , we found that the effect of such a torque depends on whether it is fixed in the star or adjusts to the reference plane formed by the angular momentum L and the reference axis, n_0 (ref. 14). In the former case, the maximum angular amplitude of the stellar wobble is $\sim 1/\Omega\tau_{\text{el}}$, where (L/τ_{el}) is the magnitude of the external torque; in the latter case, the wobble amplitude increases linearly with time, with a growth rate independent of its amplitude; the maximum amplitude is then determined by either the elastic properties of the stellar crust or by crust-core coupling which may act to damp the wobble motion. We found that in order to achieve nutation amplitudes sufficiently large to be observable (either directly or through microquakes induced by the corresponding growth in the angular part of the elastic energy stored in the crust), we require a torque fixed in the reference plane, L/τ_{el} , which is large compared to the slowing down torque, L/T .

A possible candidate for such a torque is a component of the stellar field, B_0 , which is sensitive to Ω and the actual figure axis; such a field, instead of being fixed in the star, adjusts to the reference plane and gives rise to an effective torque in the star (as viewed by a corotating observer)

$$\mathbf{N}_{\text{eff}} = -\epsilon_B I_0 (\boldsymbol{\Omega} \times \hat{\mathbf{B}}_0) (\boldsymbol{\Omega} \cdot \hat{\mathbf{B}}_0) \quad (1)$$

where ϵ_B is the 'magnetic' stellar oblateness

$$\epsilon_B = \frac{\alpha B_0^2 R^3}{4(A+B)} \quad (2)$$

and $\hat{\mathbf{B}}_0$ is a unit vector in the direction B_0 . In equation (2) α is of order unity, whereas A and B measure the gravitational and elastic energy stored in the star. (For a self-gravitating incompressible sphere, $A \simeq (3/25) GM^2/R$, and $B = (57/50) \mu V_c$ for a star of radius R with solid crustal (or core) material of volume V_c and shear modulus μ .) The torque (1) is independent of the wobble amplitude for small amplitudes; it is of order of magnitude $10^{30} \sin 2\chi$ for typical stellar fields and can, in principle, be several orders of magnitude larger than the slowing down torque; it would, however, be observable only through its influence on the wobble amplitude, which would, under these circumstances, be sufficiently large both to bring about microquakes in the star and to be directly detectable. The question then reduces to the difficult one of whether currents in the core can produce and maintain a magnetic field of this character (tied to Ω and to the shape of the crust).

Since it is sensitive to both Ω and the shape of the crust, such a field configuration must be able to slip with respect to the crust. We must therefore postulate the existence of a current sheath at the crust-core interface, which acts to decouple the field in question from the 'radiation' fields within the crust. Such screening currents are likely to appear in the star at the time the crust freezes and we are now studying ways by which this comes about.

We further note that the fields in question need not be dipolar and could well be turbulent; all that is required is that the crustal strains induced by them follow the reference plane. Under these circumstances, equations (1) and (2)

should be replaced by the suitably generalised tensor relations.

Prospects for observing such effects

Assuming that mechanisms for exciting a stellar wobble exist, the amplitude of that wobble will depend, of course, on the extent to which it is damped; here, the situation looks rather favourable for would-be wobble observers. First, in view of the likely homogeneity and sharpness of the stellar surface, the latter a consequence of the strong stellar magnetic field¹⁵, it is reasonable to conclude that no shallow oceans exist on the surface of a neutron star, so that the mechanism responsible for damping the Earth's wobble will be absent. Second, the crustal temperature of a neutron star is exceedingly low compared to its melting temperature ($T/T_m \lesssim 10^{-3}$), so that the only creep mechanism operation is the so-called logarithmic, or low temperature, creep; the time scales for the resulting plastic flow are therefore long compared to the slowing down time, T (ref. 16). Indeed, to the extent that pulsar spindown increases the elastic energy content of the crust, T provides an upper limit to the extent to which plastic flow in the crust can act to damp pulsar wobble.

Crust-liquid coupling is probably the principal source of wobble damping but this too is comparatively ineffective. From the postglitch behaviour of the Crab and Vela pulsars one knows that the time, τ , required for the crust and superfluid neutron liquid to come into equilibrium is of the order of days to years^{17,18}; such coupling gives rise to a damping time for the pulsar wobble which is far longer, being¹⁹

$$\tau_w = \frac{\Omega}{\Omega_w} \tau = \frac{2}{3} \left(\frac{A+B}{B} \right) \frac{\tau}{\epsilon_0} \quad (3)$$

on making use of the expression²⁰,

$$(\Omega_w/\Omega) = \frac{2}{3}(B/A + B)\epsilon_0 \quad (4)$$

for the free precession frequency, Ω_w . For the Crab pulsar, assuming it to have a liquid interior, $(\Omega_w/\Omega) \lesssim 10^{-7}$, so that $\tau_w \gtrsim 3 \times 10^4$ yr, at least an order of magnitude greater than the slowing down time T_{Crab} ($\sim 2,500$ yr); for the Vela pulsar, the wobble damping time is shorter than T_{Vela} ($\sim 24,000$ yr), being ~ 500 yr if the Vela pulsar is assumed to possess a solid core with $\epsilon_0 \sim 3 \times 10^{-3}$.

Given such long wobble damping times, it is reasonable to expect large amplitude stellar wobble for those neutron stars for which the effective magnitude of the exciting torque, L/τ , exceeds the slowing down torque, L/T . In such cases, the amplitude of the stellar wobble is likely limited by the maximum storage of angular elastic energy ($\propto \theta_0^2$ where θ_0 is the angular wobble amplitude) in the solid crust (or core) of the star. For the Crab pulsar, we have previously estimated that starquakes will limit the maximum wobble amplitude for a star of mass $0.25M_\odot$ to some 5° if the angular contribution to the elastic energy is comparatively small, 15° if this contribution dominates the elastic energy¹⁴. For a pulsar with a solid core, comparable wobble amplitudes may be expected; for example, if $\sigma_c/\mu\epsilon_0 \sim 1/7$, the limit on the wobble amplitude is $\sim 6^\circ$.

In their consideration of this problem, Henriksen *et al.*⁶ have assumed that the Goldreich mechanism, in which B_0 is constant in the star, is operative, and have calculated the maximum wobble amplitude for the following two cases:

(1) Stabilisation of the wobble amplitude either by internal friction in the crust or by core-crust coupling.

(2) Wobble at the elastic limit, followed by plastic flow. The estimates we have given above suggest that case (1) is probably not realised in practice; the very low temperature of the crust seems to preclude an effective internal

friction mechanism, whereas for the Crab pulsar, at least, the damping time associated with core-crust coupling is long compared to the excitation time, T , assumed by Henriksen *et al.*, so that core-crust coupling could not act to stabilise the wobble amplitude. Case (2) also does not seem realisable in practice. If only the Goldreich excitation mechanism is operative, examination of the rate of change of the oblateness and angular mismatch terms in the elastic energy shows that the oblateness mismatch contribution will grow at a faster rate than that associated with the wobble amplitude; as a result the wobble amplitude could never be large enough to induce either plastic flow or a microquake. Finally, should it reach such a critical size, we would argue that because the crust is so cold, a series of microquakes are more likely to ensue than plastic flow.

We conclude by emphasising that proposed mass and structure assignments for the Crab and Vela pulsars put definite limits on the precession frequencies one would expect to observe. Thus, for the Vela pulsar, assuming it to be sufficiently massive to possess a solid core, the corequake interpretation¹³ of its giant speedups requires an $\epsilon_0 \gtrsim 10^{-3}$; taking $B \gtrsim A$, we are led to a wobble period

$$P_w \lesssim 100 \text{ s (Vela pulsar)} \quad (5)$$

For the Crab pulsar, assuming it to have a liquid interior and a mass $> 0.3M_\odot$, the wobble period is

$$P_w \gtrsim 16 \text{ h (liquid core Crab pulsar)} \quad (6)$$

On the other hand, assuming the stellar structure of the Crab pulsar were such that it had a solid core and $(I_w/I) \sim 0.9$, one would expect that $\epsilon_0 > 10^{-3}$ and $B > A/10$, in which case

$$P_w \lesssim 5 \text{ min (solid core Crab pulsar)} \quad (7)$$

Observation of a Crab pulsar wobble could thus provide a decisive determination of its stellar structure.

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Relative positions of the 'repetitive', 'unique' and poly(A) fragments of mRNA

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The mRNA molecules from Xenopus laevis embryos represent polyribonucleotide chains of heterogeneous size transcribed from unique DNA sequences, each carrying a poly(A) stretch at the 3' terminus and at the 5' terminus, a fragment of 50 to 60 nucleotides transcribed from repeated DNA sequences.

We have previously discussed the existence of sequence heterogeneity in the mRNA molecules isolated from *Xenopus* embryos¹. On the basis of hybridisation kinetics, we concluded that each mRNA molecule was composed of a main fragment transcribed from unique DNA, with a covalently-linked smaller fragment transcribed from homologous repeated DNA sequences². It was, however, impossible to draw any conclusions about the positions that such fragments could have within each mRNA molecule.

As very similar 'repetitive' fragments seemed to be linked to different unique parts, we decided to investigate in detail the uniformity of their size and location in each mRNA molecule, also with respect to the poly(A) stretches known to be present on the majority of mRNAs.

We here present data showing that the 'repetitive' fragment is located at the 5' end of the mRNA and is 50–60 nucleotides long. We have also observed that most of the mRNAs in these embryos contain a stretch of poly(A) which is heterogeneous in size and is located at the 3' end of the molecule.

Isolation of mRNA and characterisation of the poly(A) fragment

Tritium-labelled mRNA was isolated from polyribosomes of dissociated *Xenopus* neurulae following procedures already described^{1,3}. The possible degradation of the mRNAs during extraction was already discussed and ruled out in a previous report¹ by coextracting the embryos with purified globin mRNA. Several controls also showed that significant amounts of labelled newly synthesised rRNA did not contaminate our mRNA preparation. Finally, the possibility that the ³H-uridine counts associated with the polysomes could represent cosedimenting HnRNA molecules leaked out from the nuclei is extremely unlikely in view of the fact that after only 15 min labelling, although a significant amount of newly synthesised RNA was observable, practically no radioactivity could be detected on the polysomes. It was only after longer labelling times (30 min or more) that newly synthesised RNA molecules became associated with the polysomes.

The newly synthesised mRNA sedimented on sucrose gradients as a broad peak of 12S, was very heterogeneous on acrylamide gel electrophoresis and it did not bind to Millipore

filters in 0.5 M KCl (ref. 1). Binding to nitrocellulose filters under these conditions has been reported for poly(A) containing RNAs (ref. 4) and practically all the eukaryotic mRNA investigated (except histone mRNA)⁵ have been found to contain a poly(A) stretch.

As we could have selected for molecules not containing poly(A) by our phenol extraction procedure performed in 0.1 M acetate pH 5.0 (ref. 1), we decided to re-investigate the properties of the mRNA purified from embryos labelled with ³H-adenine. The RNA was phenol-extracted in low salt and neutral pH. In these conditions, more than 90% of the radioactivity present on the polysomes is recovered after extraction and at least 60–70% of the counts are eventually found in the 12S peak on sucrose gradients. The rest of the labelled RNA sediments faster and was usually discarded because of its contamination with 18 and 28S rRNA. Millipore filters retained 25–35% of the RNA purified in this way.

To investigate the content and properties of the poly(A) associated with these RNA molecules, embryos were dissociated and labelled with ³H-adenine for 30 min or 4 h (Fig. 1). The RNA was extracted from the polysomes and digested for 30 min with RNase A and T₁. The amount of RNase-resistant material ranged between 4% and 7% of the total counts incorporated into polysomal RNA. Eighty per cent of the counts resistant to RNase A + T₁ became TCA soluble when digested with a mixture of ribonucleases T₁ + T₂, known to digest poly(A) stretches⁶. Acrylamide gel electrophoresis of the RNase-resistant material shows two distinct patterns; at 30 min (Fig. 1a) the poly(A) is distributed as the predominant peak which migrates slower than the 4S and 5S markers. The average length of the fragments in this peak is more than 120 nucleotides. The counts found consistently on both sides of the main peak demonstrate some degree of heterogeneity in the size of the poly(A) piece. At 4 h (Fig. 1b) a small amount of label is found in the 120 nucleotide peak as the poly(A) pieces are more heterogeneous in size and are distributed towards the lighter region of the

TABLE 1 Elution pattern of RNA and single stranded DNA from the HAP column before hybridisation

Phosphate molarity	% RNA eluted	% DNA eluted
0.12 M	70	100
0.14 M	13	—
0.16 M	13	—
0.20 M	—	—
0.50 M	4	—

500 µg of DNA were denatured in 0.12 M phosphate buffer for 15' at 100°C; during the cooling in ice 5,000 c.p.m. of neurula mRNA were added. The sample was then fractionated on a HAP column at 66°C.

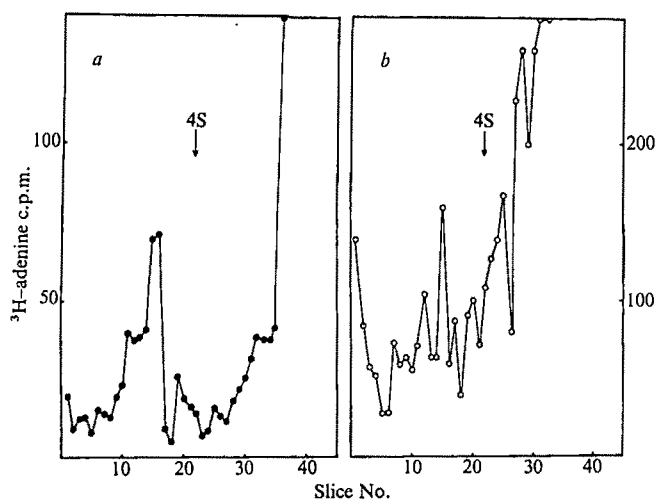


Fig. 1 Determination of poly(A) size. One thousand embryos were dissociated under the usual conditions¹. Half were then incubated in the presence of $100 \mu\text{Ci ml}^{-1}$ of ^3H -adenine for 30 min (a) while the rest were incubated under the same conditions for 4 h (b). At the end of the incubation the embryos were homogenised in sucrose-TKM buffer (35 mM Tris pH 7.4, 10 mM MgCl_2 , 70 mM KCl, 0.25 M sucrose, $7.5 \mu\text{g ml}^{-1}$ PVS and 0.5% DOC) and the polysomes prepared by sedimentation on 15–50% sucrose gradients. After extraction of the polysomes with phenol saturated with 10 mM Tris pH 7.5 and chloroform, the two samples were ethanol precipitated. The RNA was resuspended in 10 mM Tris pH 7.5, 0.2 M NaCl and incubated with $20 \mu\text{g ml}^{-1}$ of RNase A and 10 units ml^{-1} of T_1 for 30 min at 30°C . After ethanol precipitation using 1.0 absorbance unit of *E. coli* soluble RNA as carrier, the RNA was resuspended in 0.5% SDS and electrophoresed in 10% SDS acrylamide gels for 3 h at 5 mA per gel. The gels were cut into 2 mm slices, solubilised and counted. The top of the gel corresponds to the left side in the figures.

gel. Most of these fragments are lighter than the 4S marker and therefore not long enough to retain a whole RNA molecule when passed through Millipore filters. This could explain the low percentage of binding to filters that we obtain with the purified RNA.

We conclude that at short labelling times the mRNA molecules that reach the cytoplasm carry a rather long and homogeneous fragment of poly(A). When longer incubations are performed, the size of the poly(A) fragment decreases and mRNA molecules with shorter heterogeneous poly(A) fragments are detected, which seem to accumulate on the polysomes. Similar observations, which substantiate the cytoplasmic accumulation of mRNA-bearing short poly(A) stretches, have been made using the drug 3-deoxyadenosine³, known to inhibit the synthesis of poly(A) (ref. 7). There are data showing that the cytoplasmic poly(A) has a very wide distribution of sizes⁸ and that poly(A) is metabolised in the cytoplasm^{3,9,10}.

The presence of a poly(A) fragment on each mRNA molecule has also been studied, indirectly, by the use of 3-deoxyadenosine. A batch of dissociated embryos was treated for 30 min before the addition of radioactivity with $50 \mu\text{g ml}^{-1}$ of 3-deoxyadenosine. The sample was then incubated in parallel with an untreated control for 2 h in the presence of $100 \mu\text{Ci ml}^{-1}$ of ^3H -adenine. The embryos were homogenised and the polysomes prepared on sucrose gradients. Only 20% of the counts in the control polysomes were found in the 3-deoxyadenosine-treated sample. These results, obtained after long treatment with 3-deoxyadenosine, cannot be due to a specific decrease in RNA synthesis caused by drug damage to the embryos, since embryos kept as long as 2 h in $50 \mu\text{g ml}^{-1}$ of 3-deoxyadenosine and pulse-labelled with ^3H -uridine for 15 min at different times during this period showed the same amount of incorporation as the controls kept in normal saline solution. Therefore, the ap-

pearance of most of the mRNA on the polysomes must depend on the addition of poly(A) to the messenger precursor.

The poly(A) piece has been reported to be localised at the 3' end of the RNA molecule in many of the messages isolated¹¹; so we decided to determine its exact location in our system, to present a more accurate picture of the possible structure of the mRNA molecules.

For this purpose, the isolated poly(A) piece, obtained as indicated in Fig. 1, was subjected to alkaline hydrolysis in conditions that lead to at least 95% hydrolysis of the polymer to the 2', 3'-monoesters¹². The hydrolysate was then examined by paper chromatography using cold adenosine and synthetic poly(A) (also digested with alkali) as markers for nucleosides and mononucleotides. We found the radioactivity located mainly in two spots (Fig. 2). As expected, the highest counts were found in the region corresponding to mononucleotides and approximately 1% of the total counts were found in the region coinciding with the adenosine marker.

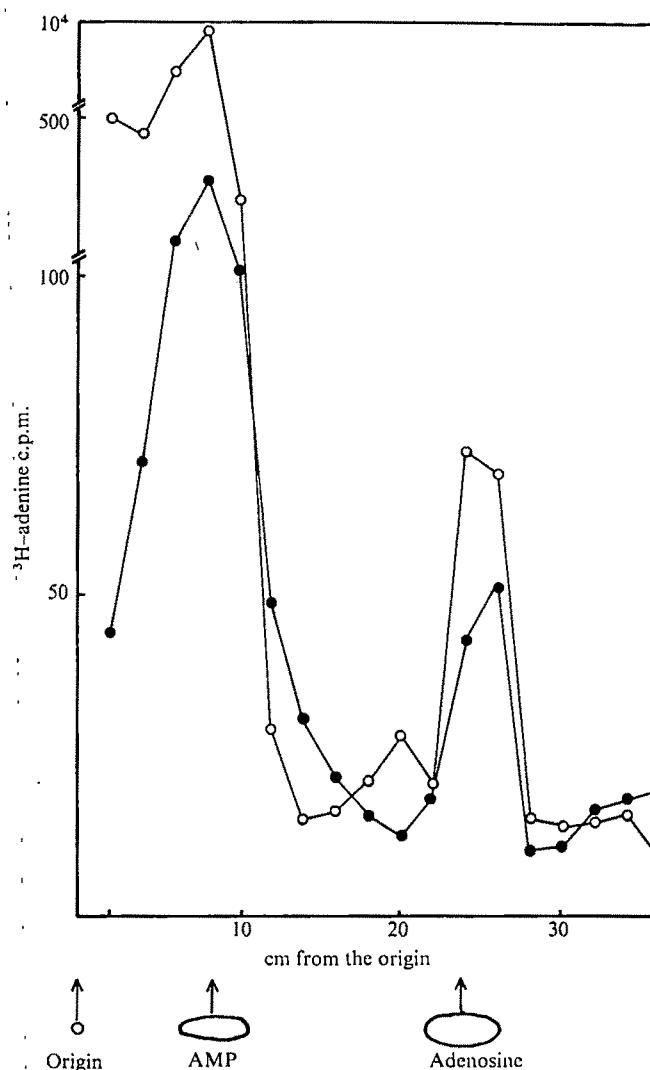


Fig. 2 Distribution of radioactivity in the alkaline hydrolysate of the poly(A) piece. The isolated poly(A) piece, obtained from embryos labelled for 2 h with ^3H -adenine, and synthetic poly(A) (Miles Laboratories, U.S.A.) were hydrolysed for 40 min at 100°C in 0.05M KOH. After hydrolysis the samples were neutralised, spotted on Whatman 3MM paper and subjected to descending chromatography in 1 M ammonium acetate pH 7.5–95% ethanol (30:75 v/v) for 10 h. A parallel paper strip containing cold adenosine was run at the same time. Paper strips were cut into 2 cm squares after exposing the spots in ultraviolet light. The paper pieces were counted in Triton-fluor mixture after digestion with 0.1M NaOH for 2 h and neutralisation with acetic acid. ●, poly(A) hydrolysate from the embryo mRNA; ○, hydrolysate from synthetic poly(A).

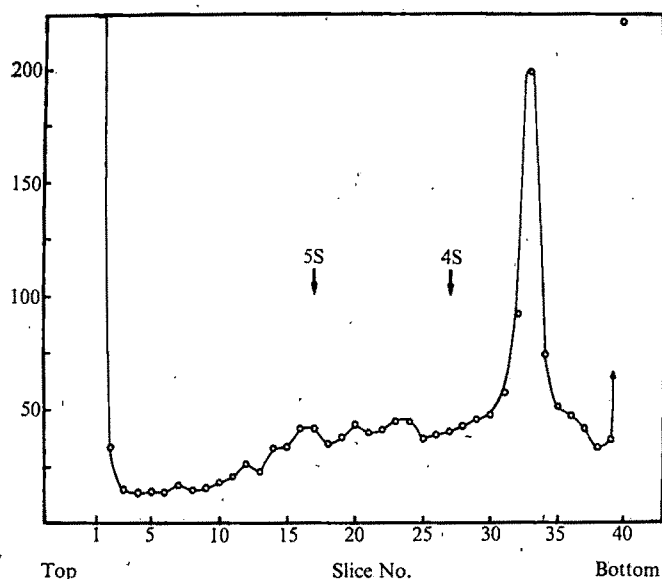


Fig. 3 Size of the isolated 'repetitive' fragment. The RNA (3,000 c.p.m.), obtained as outlined in the text, was resuspended in 0.5% SDS denatured at 100° C and electrophoresed for 3 h on 10% acrylamide gels²² tRNA and 5S RNA were used as markers. Gels were scanned in a Gilford densitometer at 260 nm and sliced into 2 mm pieces. After solubilisation in Soluene, the samples were counted for radioactivity.

The conditions used for hydrolysis do not produce significant dephosphorylation of nucleotides¹² so the nucleoside found should correspond to the 3'-OH terminal nucleotide in the poly(A) stretch. Since the enzymatic treatment does not produce a free 3'-OH end in the nuclease-resistant piece, the free OH group should correspond to the 3' end in the RNA molecule and therefore the poly(A) fragment should be localised at this end. The ratio of labelled nucleotides to nucleosides provides an average value of 75-100 nucleotides per each terminal adenosine residue in the poly(A) stretches. This value is in good agreement with the estimation from gel electrophoresis for the size of the poly(A) fragments from embryos labelled during 2 h (ref. 3).

Isolation of the 'repetitive' RNA fragment

As already mentioned, each mRNA molecule is internally heterogeneous: a part of it is transcribed from homologous repeated DNA sequences while the other is transcribed from the unique part of the genome. These two parts, called 'repetitive' and 'unique' fragments, are covalently linked and therefore present on the same mRNA molecule.

The arrangement of the sequences that we have proposed leads to two predictions: first, if each molecule carries a very similar or even identical 'repetitive' fragment, the size of the isolated part should be somewhat homogeneous. Moreover, the isolated and purified 'repetitive' stretch should rehybridise in the presence of DNA in excess, with its reiterated DNA complements reassociating completely before C_{ot} 100 (see ref. 1).

To isolate the fast hybridising part of the mRNA 120,000 c.p.m. of purified total mRNA was incubated with 30 mg of sonicated denatured DNA to C_{ot} 100. The conditions used were 0.12 M phosphate buffer and 68° C. At the end of the incubation an aliquot was withdrawn and treated with RNase (5 μ g ml⁻¹ RNase A, 30 min at 30° C). The sample was then diluted with 0.12 M phosphate buffer containing 1 M NaCl and loaded on a column of hydroxylapatite (HAP) at 66° C (ref. 13). The ratio of DNA to HAP was about 10 absorbance units of DNA (measured on native DNA before the initial melting) per ml packed HAP. The column was washed with 0.12 M phosphate—1 M NaCl until no

more DNA or radioactivity could be eluted. This step, as shown in Table 1, allows the recovery of about 70% of the RNA counts in the control, monitored before annealing. The column was then washed with increasing concentrations of phosphate buffer, and at 0.16 M the final recovery of the control RNA was 96% of the input. After annealing to C_{ot} 100 the 0.12 and 0.16 M pooled fractions containing the single-stranded DNA and the free RNA accounted for 60% of the DNA loaded and for only 5% of the mRNA counts. The 0.5 M phosphate eluate contained the renatured DNA and practically all the RNA counts. The overall yield of the column was close to 100%.

The behaviour of the RNA on the HAP column reproduces perfectly what has been obtained with nitrocellulose filters¹. The retention of the RNA by the column was 95% while the percentage of real RNase-resistant hybrids was only about 20%. We have already shown that this result is not due to artefacts or to extensive base mispairing, as the melting curves of these hybrids are very close to those of native *Xenopus* bulk DNA¹.

The most likely interpretation of this result is that the entire RNA molecule is retained because a small part of it is able to hybridise to repetitive sequences. The 0.5 M eluate was dialysed extensively against H₂O and then 0.1 M Na acetate pH 5. After ethanol precipitation the sample was resuspended in 2 \times SSC and treated with RNase to eliminate the tails of the non-hybridised 'unique' RNA. Twenty per cent of the counts were recovered and incubated with 100 μ g ml⁻¹ of pronase followed by phenol-chloroform extraction to eliminate the proteins. The DNA/RNA hybrid was concentrated, denatured by heating for 15 min at 100° C and diluted several times by addition of ice-cold 6 \times SSC. The denatured DNA was trapped on nitrocellulose filters while the free RNA fragments were recovered in the filtrate. After a short dialysis to eliminate most of the salt, the RNA was ethanol-precipitated and resuspended in different buffers according to the experiment to be performed.

The final sample contained about 50% of the initial RNase-resistant counts and about 5% DNA not retained by the Millipore filters. The purified RNA fragment was then hybridised to DNA in excess. The amount of DNA added to the reaction, together with the radioactive RNA, was about 10% of the total DNA used in each assay and therefore too low to affect the kinetics of the RNA/DNA hybridisation greatly.

The limited amount of radioactive fragments we could recover from a single mRNA preparation raised serious technical problems when we tried to measure the reassociation kinetics of the purified 'repetitive' stretch. The number of increasing C_{ot} values that could be obtained was too low to make significant measurements of the actual rate of reassociation.

Our results clearly indicate, however, that at C_{ot} 100 all the RNA input was RNase-resistant, from which we conclude that the fragments had completely and rapidly reformed hybrids with the complementary repetitive DNA sequences. These results confirm that repetitive, homologous DNA sequences are transcribed into mRNA molecules which carry also the transcript of heterogeneous unique sequences. Moreover, the 'repeated' RNA fragment can be isolated from the ' C_{ot} 100' hybrids, suggesting that the retention of mRNA by Millipore filters and HAP columns is mediated by the formation of a DNA/RNA hybrid between a family of repeated sequences and a complementary RNA fragment.

Size of the repetitive RNA fragment

The size of the isolated fragment has been measured by electrophoresis on 10% acrylamide gels. Fig. 3 shows the pattern obtained using 5S and transfer RNA as external markers; we estimated an approximate length of 50 to 60 nucleotides for the RNA stretch.

A very small amount of heterogeneous heavier material is also observed but the main radioactivity peak seems fairly homogeneous; two conclusions can thus be drawn. First, the matching of the hybrids (also reflected by the melting curves¹) must be good since RNase cannot cut within this region. It is however possible that the original hybrid might be larger than observed here and so the estimated length must be considered a minimal value. Second, the homogeneity of the fragment, as judged by electrophoretic migration, refers only to the size of the repeated RNA sequences in the formed hybrids. Taking into account the heterogeneity of the filter-binding curve¹, we cannot exclude that more than one class of repeated sequences could be transcribed into fragments of final homogeneous length.

Rapidly hybridising fragment at the 5' terminus of the mRNA molecule

To understand the biological meaning of the observed sequence arrangement in mRNA molecules it is relevant to know where the 'repetitive' fragment is located on the molecule. Since it is known that poly(A) is localised at the 3' end of the molecule, it has been possible to show that the repeated fragment is not associated with this end of the RNA.

The isolated 'repetitive' fragment is too short and homogeneous to account for the presence of any poly(A). Also, if the ³H-adenine labelled RNA is incubated with excess DNA to a C_0t 100, RNase-treated and fractionated on HAP most of the poly(A) is found in the flow through of the column, while none is associated with the retained hybrids³.

Since neither of these experiments can exclude the possibility that the RNase A could introduce a specific cut between the protected hybrids and the free poly(A) fragment, we thought it desirable to obtain direct positive evidence about the location of the rapidly hybridising fragment.

A total population of mRNAs was labelled *in vitro* with ³²P using a technique which allows specific labelling of the 5' end of the molecules^{14,15}. As all the mRNA preparations are slightly contaminated by cold tRNA and rRNA, which will also be labelled *in vitro*, a competition system was set up to prevent the hybridisation of these RNA species³. It has been shown that in excess DNA the hybridisation of trace amounts of hot RNA can be efficiently competed with by a five-fold input of cold RNA of the same species^{16,3}. The residual hybridisation obtained under these conditions is about 10%. Our experiments with ³²P-labelled RNA have been run in the presence of fifty-fold excess cold oocyte RNA. Under these conditions the hybridisation of these RNA species to a given amount of DNA (see legend of Fig. 4) should be completely prevented without causing any interference with the reassociation of the mRNA.

Figure 4 shows that when a total population of mRNA is hybridised with excess DNA¹⁷ most of the RNA hybridises to unique sequences. Only about 20% of the RNA rapidly hybridises with repeated sequences. This result confirms that the hybridisation pattern obtained in previous experiments¹ was not due to a qualitatively selected population of mRNA. In fact, the only difference we have been able to detect between total mRNA and RNA extracted with phenol at pH 5 is that the latter seems to be enriched with molecules carrying shorter poly(A) fragments.

If the same batch of mRNA is labelled *in vitro* at the 5' end and the hybridisation in excess DNA is performed (Fig. 4) monitoring the ³²P RNase-resistant counts, a completely different pattern is obtained. All the counts are hybridised at C_0t 100 and the reassociation follows second order kinetics. This should be indicative of a single reassociating species with a $C_0t_{1/2}$ of about 12–25, which in our conditions is slightly faster than rRNA. We therefore conclude that a fragment, transcribed from a family of sequences with a reiteration of about 2,000 copies per diploid genome

is present on polysomal mRNA at the 5' end of the molecule.

Previous results obtained with the filter trapping technique^{1,10}, were indicative of more than one fast renaturing component. In fact about 30% of the RNA was retained by the filters at a very low C_0t (10^{-1}) while the rest of the reaction was following slower kinetics.

The homogeneity of the hybridisation kinetics obtained with the ³²P-labelled RNA may be explained in two ways. The population of RNAs used for the filter trapping experi-

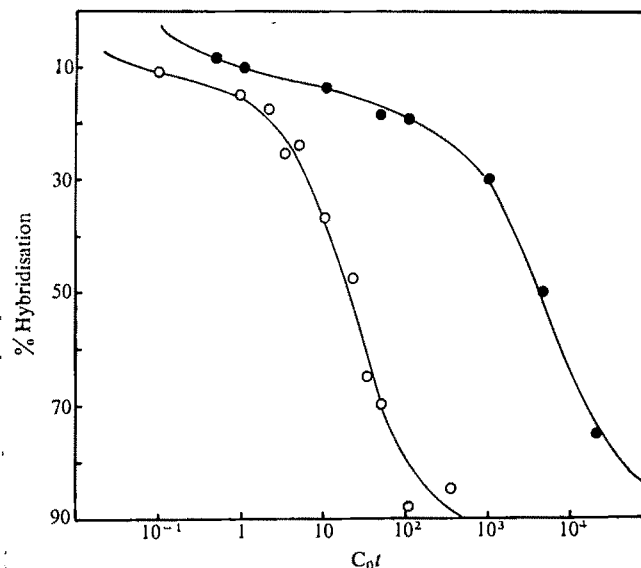


Fig. 4 DNA excess hybridisation of ³H-adenine labelled mRNA and 5' ³²P-labelled mRNA. The ³H-labelled mRNA was prepared from polysomes of 4 h labelled embryos as specified in Fig. 1. The hybridisation was performed in a great excess of DNA¹. A blank value equal to 5% of the input has been subtracted at each point as this was the RNase A resistance of the purified mRNA due to the presence of labelled stretches of poly(A). The same batch of ³H-labelled mRNA was then labelled with ³²P according to the procedure of Ilan and Ilan¹⁴. ³²P-2-cyanoethylphosphate pyridinium salt was prepared according to the method of Pfizner and Moffat¹⁵ (modified to obtain a specific activity five times as great), and was used as a donor molecule. The mRNA was treated with 10 μ g ml⁻¹ of alkaline phosphatase for 1 h at 30° C in 0.1 M Tris pH 8.2, to cleave the 5' end phosphate. To avoid degradation of mRNA, and subsequent formation of artefactual free ends, a nuclease-free preparation of enzyme was used (Boehringer, Mannheim). After phenol and chloroform extraction, the RNA was precipitated in ethanol and resuspended in dimethyl formamide. ³²P-cyanoethylphosphate in dry pyridine was added (15 ml) and the sample evaporated to dryness at room temperature. Dicyclohexylcarbodiimide (2 g) in 15 ml of pyridine was added to resuspend the mixture and the sample was incubated in a stoppered vial for 20 h at room temperature. After drying under vacuum the residue was resuspended in 30 ml water containing 1 mg of *E. coli* tRNA as carrier, centrifuged 30 min at 30,000g and the supernatant was ethanol precipitated. The RNA was resuspended in water adjusted to pH 9 with ammonium hydroxide, and the mixture was heated at 60° C for 75 min to cleave the cyanoethyl group. Before hybridisation, the RNA was further purified on a Sephadex G25 column (1.4 \times 30 cm) to remove the residual free ³²P counts. The peak of radioactivity eluted in the void volume of the column was pooled and ethanol precipitated. The pellet was finally resuspended in 0.12 M phosphate buffer. The resulting RNA sedimented, in sucrose gradients, close to the 4S marker. The yield of ³H counts was equal to about 50% of the input and the yield of ³²P was about five times greater. All the ³H and ³²P counts of this final RNA fraction became completely soluble in TCA after either alkaline hydrolysis or RNase treatment. Alkaline phosphatase treatment removed only the ³²P label. Excess DNA hybridisation was performed under the usual conditions but to obtain reproducible blanks (20–30 counts) every sample was extracted with phenol after the RNase treatment. The samples were then TCA precipitated and counted. The curve is the average of two separate experiments.

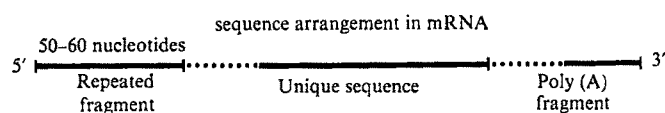


FIG. 5 A schematic mRNA molecule.

ments was extremely heterogeneous with a range of sizes varying between 6 and 18S (ref. 1). The reassociation rate of a family of sequences is strictly correlated to their size¹⁹, therefore the heterogeneous pattern of filter trapping we observed might be due to the presence of this wide variation in the size of the reassociating RNA molecules. This size effect is completely excluded using the ³²P-labelled RNA, as during the cleavage of the cyanoethyl group (see legend of Fig. 4) all the RNA is degraded to a size slightly smaller than 4S. The final hybridisation rate obtained with these fragments is slower than that for the original ³H-labelled large molecules and the homogeneity of the curve probably results from the final common length of the fragmented RNA.

Also, although most of the molecules carry a repetitive stretch at the 5' end some of them may have a second, faster hybridising part at a different position in the molecule. When the curve is monitored by the retention of hybrids on nitrocellulose filters², this second repetitive stretch could also hybridise to its DNA complements leading to the trapping of the entire RNA molecule, giving a final heterogeneous pattern of retention. This feature cannot be observed when the hybridisation is monitored by following the RNase-resistant ³²P counts, as in this case only the fragment at the 5' terminus is labelled.

The specificity of the cyanoethylphosphate *in vitro* labelling technique must be considered. In agreement with Ilan and Ilan⁴, after alkaline hydrolysis and DEAE-Sephadex chromatography we recovered, as expected, most of the ³²P counts as pXp. The presence of a small amount of monophosphates (and therefore of 3' labelling) cannot be completely excluded on the basis of this experiment since it is very difficult to purify the RNA from all the free ³²P counts which elute from the DEAE-Sephadex column in the monophosphate region. Significant labelling of the 3' end carrying the poly(A) fragment, however, does not occur, since 90% of the ³²P counts are digested by RNase under conditions which leave the poly(A) stretches intact.

The alkaline phosphatase stage in the procedure might cause some breakage of the mRNA before the *in vitro* labelling. This produces artefactual 5' ends where a phosphate could be introduced during the *in vitro* labelling procedure. The hybridisation pattern expected here, however, would be similar to the one obtained with *in vivo* ³H-labelled mRNA. It can be calculated that with only two randomly introduced breaks per molecule, approximately 70% of the *in vitro* labelled RNA would be expected to behave as 'unique'. This is certainly not the case (Fig. 4) and therefore our results clearly show that significant breakage cannot have occurred during the alkaline phosphatase treatment unless specific cuts were introduced only in the regions containing the 'repetitive' fragment. We have no way to check this possibility at present, because of the size heterogeneity of our mRNA population.

Sequence arrangement on mRNA

Figure 5 shows a diagram of an mRNA molecule based on our experimental results. Every molecule of a heterogeneous population of mRNAs is made up of three main parts. At the 5' end there is a homologous fragment, 50 to 60 nucleotides in length, transcribed from repetitive sequences. This fragment is present on every molecule and is covalently linked to a longer heterogeneous sequence transcribed from the unique part of the DNA. In agreement with reported data

for different kinds of cultured cells^{11,10} we also found evidence that poly(A) is probably added post-transcriptionally at the 3' end of the molecules. Ilan and Ilan¹⁴ reported the existence of a short homologous fragment at the 5' end of insect mRNAs; this suggests that the sequence arrangement we have observed might be a common feature of eukaryotes.

In this respect, it is relevant that experiments done directly on DNA have shown the existence of a pattern of sequence distribution which could give rise to the transcriptional products we observed. Davidson *et al.*²⁰ reported that at least 50% of the genome of *Xenopus laevis* is organised with short (300 nucleotides average) repetitive sequences intermingled with longer (1,000 nucleotides average) unique sequences. The rest of the genome also seems to be organised similarly, but with longer unique sequences interposed. As in this system, few of the intermediate steps of the mRNA maturation in the nucleus are known, it is impossible to make any strict correlation between the sequence sizes observed on the RNA molecules and the described structure of the genome.

The biological roles of the different RNA sequences carried by each molecule are so far unknown, except for the 'unique' part, which supposedly carries the information for protein synthesis. It seems important, however, that the repetitive fragment on cytoplasmic mRNA be in a specific position (5' terminus). It is possible that this sequence plays a role at the level of mRNA maturation in the nucleus and is possibly the result of the partial cleavage of one of the 'hair-pins' known to exist in HnRNA (ref. 21). A more attractive suggestion is that the homologous sequences on different messengers could be involved in translational control mechanisms.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Radio source identified with a neutral stellar object near an unusual galaxy

RECENT improvements in the accuracy of location of radio source positions to 1 arc s have made possible the reliable identification of a number of radio sources with stellar objects of neutral colour (NSO)¹⁻⁴. Two of these have the highest known redshifts, 3.4 (ref. 5), and 3.53 (ref. 6). Others have featureless optical spectra and are probably BL Lac objects⁷ (Carswell *et al.*⁸ and P. A. Strittmatter, private communication). We report here the identification of an NSO of 19 mag close to an unusual blue galaxy, using an accurate radio position obtained at 408 MHz with the Mills Cross of the Molongo Observatory.

The radio source 1107+036 was reported by Munro⁹ and has been identified with a nearby blue galaxy of 17 mag¹⁰ (for reasons explained below) rather than with the NSO of 19 mag noted to the west and closer to the radio position. Figure 1 is an enlargement of a section of prints from the National Geographic-Mount Palomar Sky Survey, which clearly shows both objects. The radio positions in ref. 10 were accurate to ~ 5 arc s and the total number of NSOs within the radio error regions was consistent with chance expectation. Radio and optical positions accurate to ~ 1 arc s are necessary to identify an NSO reliably. A position of this accuracy has now been obtained at Molongo for 1107+036. The improvement principally arises from repeated observations during special calibration sessions, in which a large number of sources with known optical positions are observed on successive days, and other objects of interest are interspersed with these. The main purpose of these sessions is to discover and monitor time-varying sources¹¹ and to test the performance of the aerial.

The radio source 1107+036 was observed on six different days spanning three different calibration sessions. The use of numerous calibrators close to the source and the repeated observation of the source enables the rejection on statistical grounds of occasional results affected by severe ionospheric or meteorological conditions. The result is presented in Table 1 together with the optical position obtained from the sky survey prints. A new optical measurement for the NSO is consistent with that already reported¹⁰ and the mean of the two measurements is given in Table 1.

The identification is highly likely because the probability of a chance association at a separation of 2.1 arc s is about 0.004, based on star counts¹⁰ in the appropriate range of galactic latitude.

The source does, however, differ in one important respect from most NSOs identified with radio sources, in that it has a steep radio spectrum with spectral index 0.99 ± 0.07 . This is a revision of the value quoted by Murdoch and Hoskins¹² and uses the present flux density at 408 MHz of 1.77 ± 0.05 Jy (1 Jy = 1 f.u.). Extrapolation to 178 MHz would indicate at flux density of 4 Jy but the source does not appear in the 4C catalogue¹³. This could indicate self absorption of low frequency but is probably a result of the nearby occurrence of 1108+034 (4C03.21) and 1107+045 (4C04.35). Most radio sources identified with NSOs have 'abnormal' radio spectra¹⁻⁵, (where the term normal implies a reasonably

straight radio spectrum with spectral index steeper than -0.5). It is not clear, however, to what extent the predominance of abnormal spectra may be a selection effect which arises because most of the accurate positions necessary for the identification of NSOs have been obtained at high frequency.

The nearby blue galaxy is an interesting object in its own right. There is an extensive blue envelope of diameter ~ 20 arc s which is apparently non-uniform. The most prominent features are a blue condensation to the north and, on the red print, a curved feature extending outwards from the central region toward the south-east. A high resolution photograph is necessary to test the reality of these features.

The original identification was made with the galaxy¹⁰ despite its displacement outside the error region, because it was found that the total number of galaxies beyond the radio error regions, but within 20 arc s of the respective radio positions, was significantly in excess of chance expectation. Among the various cases in this category, 1107+036 seemed to be one of the more likely cases of a genuine displaced identification. Now that an alternate identification has been found, the question arises as to whether the nearby presence of the galaxy should be regarded as coincidental, or whether the two objects might be associated.

We might question whether all of the galaxy identifications suggested by Hoskins *et al.*¹⁰ with radio-optical separations greater than the error limits might be coincidental. This probability was assessed by them to be 0.007. We note incidentally that Véron and Véron¹⁴ have suggested that radio-optical separations are very rare. There is, however, further supporting evidence for such radio-optical separations for galaxies when measurements are made with the 2.9-arc min beam of the Mills Cross. Separations up to ~ 30 kpc were common for galaxies in Abell clusters¹⁵. Separations of ~ 40 kpc from the radio centroid have been found for galaxies with low-brightness extended components¹⁶. We do not, therefore, believe that all the suggested identifications involving a radio-optical separation should be rejected.

There are two sources apparently similar to 1107+036 that warrant brief mention. The variable radio source 0048-097 has recently been identified⁴ with an NSO, in place of the original identification with a nearby blue galaxy¹⁷, and is

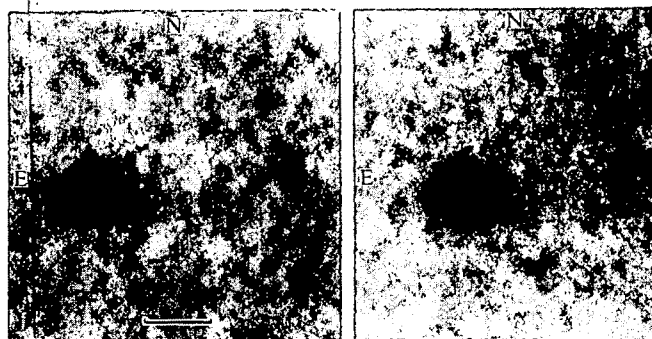


Fig. 1 Red and blue images of the NSO 1107+036 and the nearby galaxy, enlarged from the National Geographic-Palomar Survey prints. The scale marked on the red print is 20 arc s long.

TABLE 1 Molonglo positions for NSO1107 + 036

Radio 408 MHz	11 h 07 min 49.38 \pm 0.07 s	03°37' 52.8 \pm 1.5"
Optical NSO	11 h 07 min 49.25 \pm 0.03 s	03°37' 53.6 \pm 0.4"
Galaxy	11 h 07 min 50.51 \pm 0.04 s	03°37' 56.3 \pm 0.6"

probably a BL Lac object⁷. In this case the NSO is brighter than the galaxy. The second case is 0837+242 (4C24.18) which was identified with a nearby irregular blue galaxy (17. mag, ref. 18) rather than with a stellar object closer to the radio source. Burbidge¹⁹ found a continuous optical spectrum for the stellar object which has been described as blue^{14,18} and as neutral¹⁹. Véron and Véron¹⁴ consider the stellar object to be the identification but J. M. Sutton (private communication) has obtained a more accurate radio position which is 13 arc s from the stellar object. The identification is therefore seriously in doubt.

The one additional case of an NSO within 20 arc s of a blue galaxy is not a convincing argument for associating the blue galaxy with the radio source in either case (0048-097 or 1107+036). In the absence of direct evidence, we consider that further cases need to be found before the association can be justified statistically.

The general nature of neutral stellar objects is not clear, but it is suspected that they are linked to the BL Lac objects (Carswell *et al.*⁸, and P. A. Strittmatter, private communication). Racine²⁰ has suggested that BL Lac (and hence presumably other objects in this class) may represent a particular type of galaxy closely related to N galaxies and Seyfert galaxies. Among other bright BL Lac objects and possible BL Lac objects 1727+50 (ref. 7) was listed as a very compact galaxy²¹, 1101+38 (ref. 22) was listed as an extremely compact galaxy²¹, and AP Lib (1514-24) was classified as an N galaxy²³.

In fact, a problem in classifying the optical counterparts of radio sources is that any morphological classification depends directly on apparent magnitude (and therefore, presumably, distance). It may well be that the NSOs currently being identified are fainter members of the same class of compact objects. The occasional nearby presence of other galaxies would then no longer be surprising because of the known tendency of galaxies to cluster.

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Search for high frequency optical variations in Vela XR-1

THE binary system associated with the 6.87-mag B0.5Ib star, HD 77581, which is identified^{1,2} with the Vela X-ray source, 2U0900-40, is a good candidate for optical studies with high time resolution because of its relative brightness. A 28-min data set consisting of consecutive integrations of 1 ms was taken on the night of February 25-26, 1973 with the Cerro Tololo 152-cm telescope using an S-20 photomultiplier with no filter. Analyses using power-spectral and cepstrum techniques³ show no significant activity at a level greater than 0.25% in the range from 0.06 Hz to 500 Hz (except for instrumental artefacts at 60, 180, 300 and 500 Hz). No aperiodic variations were detected to a weaker limit (1%-2%); at low frequencies (<10 Hz) increased variance ascribable to atmospheric scintillation was present. This result was confirmed by a separate analysis of about 15 min of data from an observation of 1 h on the night of January 13-14, 1973. That the X-ray emission is actually associated with a degenerate, collapsed (and perhaps invisible) companion to the brilliant supergiant, however, makes a stronger limit very desirable.

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Alaska to New Zealand whistler-mode transmission at 6.8 kHz

OBSERVATIONS of whistler-mode signals from v.l.f. communications transmitters have been used^{1,2} to study the magnetosphere in a way similar to the use of naturally occurring whistlers from lightning. Existing naval communications transmitters provide high radiated power (up to 1 MW) but are restricted to transmission at allocated frequencies mostly above about 15 kHz, are not well located geographically for this work and are not often available to transmit special programmes.

We report here one result of an experiment for which we set up a transportable v.l.f. transmitter in Alaska and a receiving station in New Zealand located so as to be approximately geomagnetically conjugate. Since the Otago group already had v.l.f. receiving facilities at Dunedin, New Zealand, the transmitter was set up (by the Aerospace group) at Port Heiden, Alaska, about 200 km from Dunedin's conjugate. The transmitter antenna was a vertical monopole lifted to 1,000 to 1,500 m by balloon. The transmitter and all associated equipment (including power generator) are mounted on trailers so that the installation can be set up or removed in a matter of days. Although transmitter location and frequency are quite flexible, radiated power obtainable is quite low. This varies from a few kW at 21 kHz to about 100 W at 7 kHz. During the event described here the radiated power was only 13 W.

The event occurred about 1 h after local midnight on (Greenwich date) August 27, 1972. The transmitter operated at 6.8 kHz (at 13 W) from approximately 1307 to 1342 GMT. Whistler-mode signals were first detected at Dunedin at about 1324 GMT. Signal strength increased with some variation to a maximum of $35 \mu\text{V m}^{-1}$, before fading again to below detectability at about 1330 GMT.

During this period of good reception, the transmitter was keyed on for 0.5 s in each second from 1326 to 1327:41 and then continuously ("c.w.") to 1329:10 GMT. Before 1326 and after 1329:10 the transmitter sent pulses of varying length and separation. From these the one-hop delay was measured as 1.13 ± 0.01 s. Maximum signal strength ($35 \mu\text{V m}^{-1}$) occurred between about 1328:20 to 1328:40 during c.w. transmission. A short section is shown in Fig. 1.

Twelve whistlers were received at Dunedin during the period 1325 to 1330. Most reached up to 8 kHz and were strongest between 5 and 6 kHz. Eight of these whistlers were 'short' or one-hop and the other four were 'long' or two-hop. None showed echoes beyond 2-hop, or any associated hiss or triggered emission, or any other evidence that duct amplification might be higher than typical.

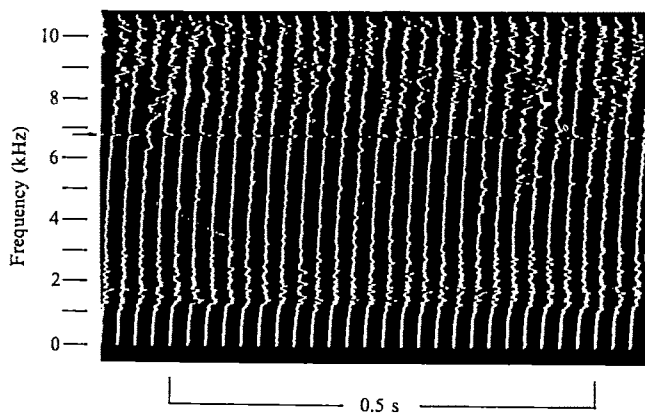


Fig. 1 Dynamic spectrogram of a short section near 1328:30 UT, using a Rayspan scanning at 48 sweeps s^{-1} . Trace separation corresponds to about $40 \mu\text{V m}^{-1}$. Signal at 6.8 kHz is indicated by arrow.

Although no whistler reached the nose frequency, dispersion analysis using the "linear-Q" technique³ showed that all whistlers travelled in the one duct at $L = 2.92 \pm 0.04$ (invariant latitude 54.2°) with one-hop delay at 6.8 kHz of 1.13 ± 0.01 s. From this it can be safely assumed that the signals from our transmitter travelled in this duct also.

An estimate of the duct amplification can be made by comparing the observed signal strength with that calculated for no duct amplification. Helliwell provides analytic expressions and graphs for making such calculations on pages 64 to 81 of ref. 4. Briefly his method involves calculating the field of the wave transmitted into the duct, the effective cross section, trapping and transmission efficiency of the duct, the field at the duct output (Dunedin end), the absorption through the D, E and F region, and finally the waveguide loss from the duct exit region to the receiver (Dunedin). Although we know the duct latitude by dispersion analysis, we do not know duct longitude and hence the distances Port Heiden—duct entrance and duct exit—Dunedin. If the duct were fortuitously located to minimise these distances (100 km and 160 km respectively) the signal expected would be about $1.5 \mu\text{V m}^{-1}$ to within about 3 dB. The observed signal strength of $35 \mu\text{V m}^{-1}$ thus implies amplification of about 27 ± 3 dB for optimum duct longitude. Higher amplification would be needed for other duct longitudes. Thus if the duct exit had been a "reasonable" 1,000 km from Dunedin, an additional 20 dB of amplification would be required.

Since the observed whistlers were typical and unremarkable, we conclude that duct amplification at night is typically of the order of 30 dB per hop. As a consequence whistler mode signals can be obtained from quite low power transmissions at 'low' (~ 7 kHz) frequencies.

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Effect of atmosphere and ionosphere on magnetospheric micropulsation signals

THE large-scale structure of low frequency hydromagnetic waves in the magnetospheric plasma can be understood from observations of micropulsations at the Earth's surface. Signals have to penetrate the ionosphere and atmosphere and although the scale heights of these layers are much less than the scale lengths expected for the magnetospheric phenomena, the behaviour is not as simple as might be expected^{1,2}.

Recent computations³ (also, W. J. Hughes, to be published) allow an analytical approach to the problem.

Dungey¹ split the disturbance seen on the ground into two parts, one part with zero vertical current at the ground and the other with a vertical current. The latter part behaves differently from the former and is in fact strongly screened by the ionosphere. The vertical current at the ground is non-zero and this part of the disturbance therefore has a vertical electric field associated with it, given by

$$\frac{1}{c} (4\pi\sigma + i\omega) E_z = (\text{curl } \mathbf{b})_z$$

where \mathbf{b} is magnetic disturbance and σ is the atmospheric conductivity. Therefore, near the ground

$$E_z \sim \frac{cb_g}{(4\pi\sigma_g + i\omega)l} \quad (1)$$

where l is the horizontal scale length of the disturbance. This vertical electric field is related to the horizontal electric field by Faraday's law so that, also near the ground,

$$\frac{\partial E_y}{\partial z} - \frac{\partial E_z}{\partial y} = \frac{i\omega}{c} b_g \quad (2)$$

where y is the direction of horizontal variation of the micropulsation magnetic field. Using equation (1) in equation (2), together with the approximation

$$\frac{\partial E_z}{\partial y} \sim \frac{E_z}{l}$$

it is clear that if

$$\frac{(4\pi\sigma + i\omega)\omega l^2}{c^2} \ll 1 \quad (3)$$

then

$$\frac{\partial E_z}{\partial y} \sim \frac{\partial E_y}{\partial z} \quad (4)$$

and the disturbance is effectively electrostatic in the atmosphere. Equation (3) is satisfied for $\omega \sim 0.1$ – 0.01 s⁻¹ by any non-uniform disturbance on the Earth.

It follows that because the electric current is divergence free and the conductivity rises markedly with height, E_z decreases rapidly with height. Equation (4) shows that the change of E_y with height is proportional to E_z and, as the numerical integrations show, with any reasonable atmospheric model the horizontal electric field is more or less constant from a height above 20–30 km and will be of the order $h E_{zg}/l$, where h is the scale height for conductivity (and thus for E_z).

This electric field drives both Pedersen and Hall currents in the ionosphere and the increase in magnetic field resulting from the currents, in both the x and y directions, is given by Ampere's Law and is of the order

$$\begin{aligned} \Delta b &\sim \frac{4\pi}{c} \Sigma E_y \\ &\sim \frac{4\pi}{c} \Sigma \frac{h E_{zg}}{l} \\ &\sim \frac{4\pi}{c} \Sigma \frac{h}{l} \frac{cb_g}{(4\pi\sigma_g + i\omega)l} \end{aligned}$$

where Σ is a height integrated ionosphere conductivity. For $\Sigma \sim 10^{13}$ electrostatic units (e.s.u.) $h \sim 10^6$ cm, $l \sim 10^8$ cm and $\omega \sim 10^{-1}$ s⁻¹ the field at the ionosphere is $\sim 10^5$ greater than that at the ground. This part of the disturbance is thus effectively screened from the ground by the ionosphere.

The other part of the disturbance, that is, the part with $(\text{curl } \mathbf{b})_z = 0$ at the ground, behaves more simply. Everywhere at the ground $E_z = 0$ and so the horizontal electric field and magnetic field are related by

$$\frac{\partial E_z}{\partial z} = \frac{i\omega b_{yg}}{c}$$

or

$$E_z \sim \frac{i\omega}{c} z b_{yg}$$

Computations show that this approximation is valid up to the top of the ionosphere. The change in magnetic field produced by the ionospheric current is of the order

$$\Delta b \sim 4\pi b_g \frac{\Sigma d\omega}{c^2}$$

where d is the mean height of the high Pedersen or Hall conductivity region in the ionosphere. For $\omega \sim 10^{-1}$ and $d \sim 10^7$ cm, Δb is of the order of the magnetic field at the ground. At lower frequencies ($\omega \lesssim 10^{-2}$) the ionospheric contribution to this part may be ignored, and the magnetic fields above and below the ionosphere are the same.

As the ionosphere screens only that part with $(\text{curl } \mathbf{b})_z \neq 0$, then $(\text{curl } \mathbf{b})_z$ must be effectively zero for any disturbance seen on the ground, whatever the disturbance in the magnetosphere. The magnetospheric disturbance is determined by the hydromagnetic wave equations and boundary conditions very far from the Earth. A magnetospheric disturbance will generally contain parts of each disturbance described, and thus will not be totally screened.

In a cold plasma (which is a good approximation for most of the magnetosphere away from the equatorial region) there are two wave modes; the isotropic fast mode and the transverse mode which is guided by the magnetic field. The scale length of variation for the isotropic mode is $2\pi A/\omega$ where A is the Alfvén speed. For $\omega \sim 10^{-1}$ – 10^{-2} s⁻¹, and an Alfvén speed of the order of 10^8 cm s⁻¹, the scale length of the fast mode is much greater than terrestrial dimensions. This implies that if the disturbance is localised on the Earth, the fast mode contribution is evanescent and the vertical scale of variation of the disturbance in the magnetosphere is comparable to its horizontal scale on the ground. If boundary conditions are then applied between the ionosphere and the magnetosphere for horizontal scales of about 300 km or greater, the disturbance on the ground is proportional to the transverse mode amplitude in the magnetosphere.

Observations of the localisation in latitude of an event at a given micropulsation frequency^{4,5} suggest that the field line resonances of the transverse hydromagnetic mode are an important source of micropulsations.

At mid and high latitudes, however, where the dip angle is large, the transverse mode has $\nabla \cdot \mathbf{b}_{\text{HOR}} \approx 0$ in the magnetosphere. But on the ground the ionospheric screening ensures that only the part with $(\text{curl } \mathbf{b}_g)_z \approx 0$ will be seen. If the horizontal scale is great enough for the magnetospheric and ground magnetic fields to be directly proportional, then the ionospheric currents must act so as to rotate the horizontal vector of the magnetic field through 90°. In such a case the horizontal polarisation pattern seen on the ground needs to be rotated through a right angle to obtain the magnetospheric pattern.

Features of a disturbance which exhibits a peak at a fixed latitude and a periodic variation in longitude, can be simply outlined. At the latitudinal peak the disturbance should be linearly east-west polarised on the ground. Elsewhere the ground polarisation will be elliptical with the major axis north-south for events which are strongly lo-

calised latitudinally. This result is in agreement with some earlier experimental observations⁵⁻⁸.

In conclusion, the properties of micropulsation signals discussed here seem to be an important feature of at least one class of pulsations; and our studies suggest that to a first approximation the polarisation ellipse on the ground should be rotated through a right angle to obtain the magnetospheric polarisation.

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Middle Cretaceous sediments from the eastern part of Walvis Ridge

THE Walvis Ridge is one of the most conspicuous features of the South Atlantic. It extends from near Tristan da Cunha on the Mid-Atlantic Ridge to the African continental margin and has three main segments. The western segment is oriented SW-NE, the central one N-S and the eastern one SSW-NNE. The Walvis Ridge may have kept pace with the opening of the South Atlantic which started in the Early Cretaceous¹⁻⁸, either by transform fault mechanisms^{7,8} or by a mantle hot spot and plume⁹⁻¹². The eastern segment

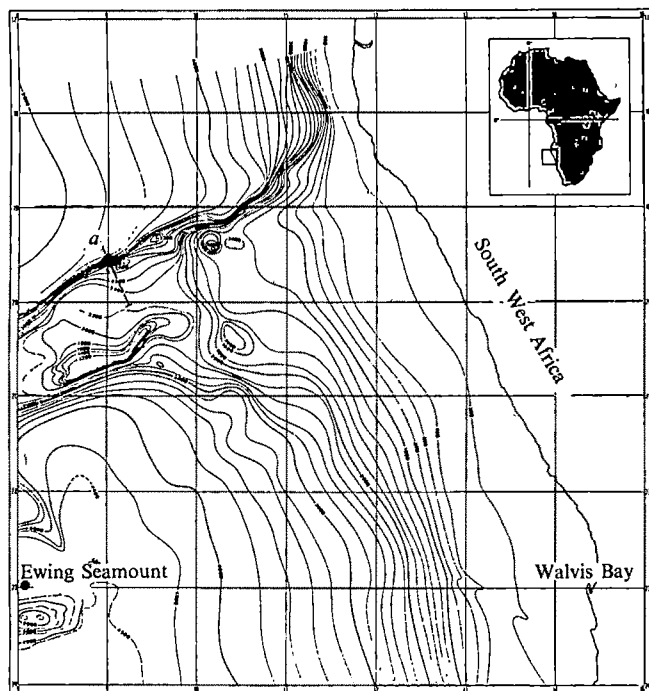


Fig. 1 Bathymetric map of the eastern segment of the Walvis Ridge showing the location of the seismic reflection profile shown in Fig. 2, and the locations of the sediment samples. a, Site of dredge. Depths in corrected metres. Contours at intervals of 200 m.

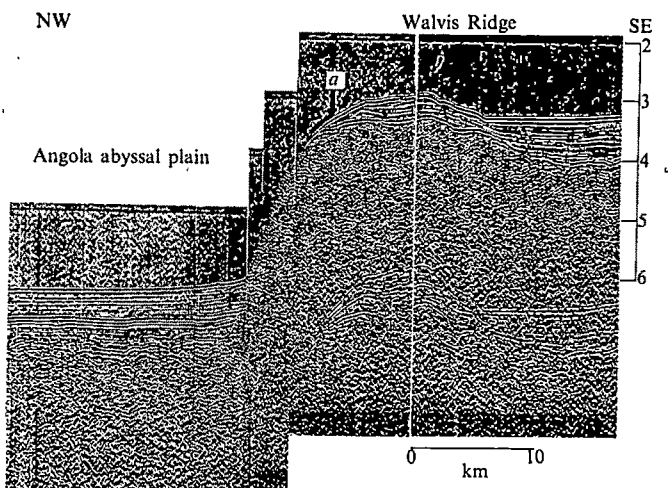


Fig. 2 Seismic reflection profile across the northern scarp of the eastern segment of Walvis Ridge and location of the dredged rocks. a, Site of dredge.

is thought to have been built throughout the Middle Cretaceous, that is, between 120 m.y. ago and 80 m.y. ago¹³⁻¹⁵. We report information obtained from an investigation of the age and depositional conditions of sedimentary rock recovered during the Walda cruise of the RV Jean Charcot (April-August 1971).

White chalk fragments containing numerous fossils were recovered from a depth of 2,700 m by a dredge (CH 18 DR 04) on the northern flank of the Walvis Ridge in the centre of the eastern segment (19° 33'S; 09° 01'E) (Fig. 1). Smear slides of the calcareous matrix show mainly coccoliths and micritic calcite. Glauconite and biotite are also present. The most abundant macrofossils are gastropods, remains of echinoderms (regular and irregular urchins: *Holasteroidea*, *Spatangoida*) and bivalves (including *Ostreidae*, *Arcacea*, *Heterodonta* and *Neithea*). One specimen of *Neithea* could be *Neithea cf. shawi* Pervinquière (= *N. coquandi* Péron). The distribution of *N. shawi* is Albian-Cenomanian in the Angola Basin but mostly Cenomanian in northern Africa¹⁶. This specimen is, however, poorly preserved and could belong to the species *N. regularis* Schlotheim. In Europe the distribution of *N. regularis* Schlotheim ranges from Turonian to Senonian (Freinex, personal communication). Thus the macrofossils indicate an Albian-Senonian age. The nannofossils consist mainly of *Lithastrinus floralis* Stradner, *Cricolithus multiradiatus* Kamptner, *C. pemmatoideus* Deflandre, *Loxolithus armilla* (Black) and *Parhabdololithus embergeri* (Noël). These are typical Albian-Cenomanian species. The sediment is therefore tentatively dated Middle Cretaceous, that is, about 100 m.y.BP. This agrees with ages proposed by other authors¹³⁻¹⁵.

Macrofossils are not common in a coccolith ooze. Hypotheses for a depositional environment of the sediments, which might explain the observed association include (1) the coccoliths accumulated in deep water and the macrofossils are thanatocoenoses transported from shallow depths by turbidity currents or some other process; (2) both the coccoliths and macrofossils were deposited in shallow water. We favour the second hypothesis, tentatively rejecting a mixed origin for the sediments because of the distance from the African coast and the absence of local seamounts. A shallow water environment would comply with the ecological needs of the macrofossils. *Neithea*, for example, is commonly found in shallow water Albo-Cenomanian deposits in the Angola Basin¹⁶. Furthermore, coccoliths are epipelagic organisms and cannot be used as precise depth indicators¹⁷. Although they are typical of open sea sedimentation, their occurrence does not necessarily imply deep water.

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Geological map of the Horn of Africa and surrounding regions, showing tectonic features and geological formations. The map includes labels for countries like Sudan, Ethiopia, Kenya, Somalia, Djibouti, and Arabia, as well as major bodies of water like the Red Sea, Gulf of Aden, and Indian Ocean. Key geological features include the Afar Depression, Lake Tana, Lake Victoria, and the Belet Wen Fault Zone. A legend in the top right corner details the geological formations and tectonic features, including Mesozoic, Eocene, and Miocene sediments, and recent faulting. A scale bar at the bottom left indicates distances up to 300 km.

Legend:

- Formations unaffected by important Mesozoic - Recent faulting:
 - Pliocene - Recent sediments
 - Eocene - Miocene sediments
 - Eocene - Miocene volcanics (Trap series)
 - Mesozoic sediments and Precambrian
- Regions affected by Mesozoic - Recent faulting:
 - Miocene - Recent rifting
 - Jijiga - Belet Wen Fault Zone
 - Mekele Faulting
 - Pliocene - Recent faulting in Afar parallel to older trends
 - Mekele trend in central Afar
 - Jijiga - Belet Wen trend

FIG. 1 The geology and large scale structure of the area around the Afar Depression.

The faults affect at least the lower part of the Trap Series basalts, which are considered to be mainly of Oligocene-Miocene age in southern Ethiopia⁶. The intercalation of the lowermost basalt flows with Cretaceous(?) sandstones near Jijiga⁶ does, however, raise difficulties in the assignation of a precise lower age limit for the faulting. A further consideration here is that the main period of faulting could have preceded the basalts. An upper age limit for the faulting is obtained from the observation that they are cut by the

ENE-trending faults of the southern margin of the Afar Depression, which are of Lower–Mid Pliocene age⁷.

Tectonic conditions in the Afro–Arabian region have changed considerably with time. In the case of the Mekele–Northern Somalia movements there is a long time gap before later tectonic activity and we follow Sowerbutts⁸ by suggesting that this is a graben structure related to a Cretaceous Gondwana plate separation (India–Africa?), and that it is not merely an early phase of the present Afro–Arabian movements.

The tectonic relationships of the Jijiga–Belet Wen faulting are more problematical. Movement was possibly dextral, accommodating early plate separation across the Gulf of Aden, unaccompanied by Red Sea movements. This view is in direct contrast to that of Burek⁹ who suggested that such early opening of the Gulf of Aden was accommodated by sinistral transcurrent movement along the present Red Sea, and considered the Harar–Somali plateau to be a single rigid unit. The Gulf of Suez is a key area here. If the transcurrent faulting postulated by Youssef¹⁰ and others is substantiated, our work now suggests a third and more exciting possibility: an independent lower–Mid Tertiary transcurrent movement along the entire Suez–Red Sea–Jijiga–Belet Wen line which determined the locus of later plate separation. In Afar the ‘oceanic’ Erta’Ale volcanic range¹¹ is perfectly aligned on the Jijiga–Belet Wen structure.

It is also clear that planes of weakness along these structures, and the Mekele faulting, have locally controlled the detailed trend of later faulting within Afar. NNW–SSE (Red Sea trend) faulting occurred in the Aisha area north and north–northwest of Jijiga both before and after extrusion of the post-rifting Pliocene flood basalts¹² although this trend of faulting is uncommon elsewhere in southern Afar. Similarly WNW ‘Mekele trend’ faulting is seen in both pre-Tertiary rocks and in Pliocene flood basalt terrains in the southern Danakil Alps and in the Assab–Tendaho region¹³, where diamond-shaped interference patterns occur between WNW and NNW (Red Sea trend) faulting. The reappearance of these fault trends cutting Pliocene flood basalts lends further support to the view that substantial regions of the Afar are underlain by attenuated continental crust.

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Tin mineralisation above mantle hot spots

MANY important tin belts of Phanerozoic age, including those in Bolivia, Mexico, Alaska, Malaysia–Indonesia, eastern Australia and western Europe, are believed to have formed above subduction zones at convergent plate margins^{1,2}. If the late Tertiary mineralisation in the southern part of the Eastern Cordillera of Bolivia is representative of this type, then the mineralisation occurred above the deepest parts of subduction zones with the tin perhaps related to partial fusion of subducted oceanic lithosphere³.

In addition to tin deposits at active or inactive convergent plate margins, and Precambrian tin-bearing pegmatites (for example, Congo and Western Australia), there is a third genetic type of economically important tin deposit, which is discussed here. This type formed away from lithospheric plate boundaries, in intraplate environments, and is represented by major deposits on the Jos Plateau in Nigeria^{3,4} and in Rondônia and adjoining states of southern Amazonia, Brazil^{5,6}. Smaller deposits occur in the Air and Zinder areas of Niger⁷, at Mayo Darlé in Cameroun⁸, in the Hoggar massif of southern Algeria⁹ and its extension into the Adrar des Iforas in Mali, at Sabaloka in northern Sudan¹⁰, in south-eastern Egypt¹¹, in the Tibesti area of Chad, in southern Morocco, and in the Damaraland province of South-west Africa^{11,12} (Fig. 1). Tin deposits in Transbaikalia and Mongolia are also of this type (N. Varlamoff, personal communication, 1973).

All of these deposits are associated with small, alkaline and peralkaline granite plutons of anorogenic character—the Younger Granites of Africa—that cut discordantly through the basement which is commonly of Precambrian age. The important Nigerian^{3,4} and Rondônia^{5,6} examples are typical and occur in circular to oval volcanic–plutonic complexes 5 to 20 km wide. These are characterised by early rhyolitic volcanics that are succeeded by multiple granitic intrusives, the emplacement of which was commonly controlled by ring fracturing and cauldron subsidence. Basic and intermediate rocks are also present at some centres. The granites and their porphyritic equivalents are rich in fluorine, containing accessory fluorite and topaz. Mineralisation is in the form of cassiterite accompanied by topaz, lithia mica and some wolframite in greisens and swarms of quartz veins in the roof zones of biotite granites. The complexes in certain areas, such as Hoggar¹³ and Transbaikalia–Mongolia, lack ring structures and may represent a deeper level of emplacement. Although mineralogically similar, the tin deposits in South-west Africa are somewhat different since many occur in pegmatites; at one locality, however, a stockwork of quartz veins is mined^{11,12}.

The tin-bearing, subvolcanic complexes occur in variously oriented lines, several of which constitute elongated belts, approximately 300 to 400 km long, in Nigeria, Rondônia, South West Africa (Fig. 2) and elsewhere. Progressive age trends along the lines of complexes have been claimed^{14,15} but are not strongly supported by the available radiometric data^{16,17} (Fig. 2).

Radiometric dating has demonstrated emplacement of the stanniferous complexes over a protracted time interval: late Precambrian (around 1,000 m.y. ago) in Rondônia^{18,19}, Cambrian in the Hoggar⁹ and at Sabaloka in Sudan¹⁰, Carboniferous in Air²⁰, Jurassic in Nigeria^{21,15}, Jurassic and Cretaceous in South West Africa²², Cretaceous in Trans-

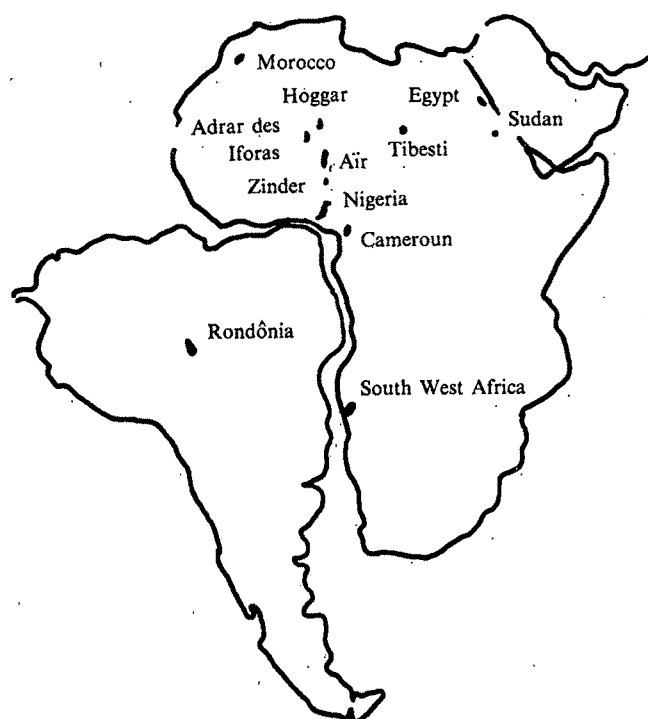


FIG. 1 Areas of tin-bearing, alkaline granitic complexes plotted on a pre-drift reconstruction of Africa and South America. The tin-bearing complex in Cameroun was, however, emplaced after the inception of spreading in the South Atlantic in the early Cretaceous²⁴.

baikalia-Mongolia (N. Varlamoff, personal communication, 1973), and Eocene at Mayo Darlé in Cameroun⁸.

The concept of hot spots or mantle plumes was introduced by Morgan^{23,24} after the earlier ideas of Wilson²⁵. Hot spots are considered to be manifestations of solid-state convection in the lower mantle, and consist of vertical columns or plumes of ascending primitive mantle material. Within the asthenosphere the plumes spread laterally^{23,24}, producing, in some cases, rifting and consequent separation of plates of continental lithosphere²⁶. The fixed mantle plumes are considered to 'burn' through lithospheric plates, thus giving rise to localised igneous activity. At the outset, when the lithosphere is continental, this activity is represented by alkaline magmatism including alkaline granitic, subvolcanic complexes^{14,15,26}, whereas once oceanic crust has been generated the plumes give rise to basaltic oceanic island chains or to aseismic ridges^{23,24,25}.

I therefore propose that the tin-bearing, alkaline granitic complexes in Nigeria, Rondônia and elsewhere were generated above mantle hot spots during a number of brief intervals since the late Precambrian. In all cases the effect of the hot spots seems to have been limited to domal uplift and the emplacement of linear belts of complexes, and activity apparently ceased before the inception of the rifting stage proposed by Burke and Dewey²⁶. In many cases the plumes sought out fundamental breaks in the continental lithosphere and these now seem to control the location and linear array of the stanniferous complexes^{16,17,20,27}. The extents of the tin-bearing belts (Fig. 2) are presumed to reflect the dimensions of the hot spots that produced them and not to indicate widespread migration of magmatism resulting from differential motion between the plumes and the overlying lithosphere^{14,15}.

Economic concentrations of tin are traditionally thought to have been derived from the continental crust (see ref. 28). The concept of tin mineralisation above rising plumes of mantle material suggests, however, that the mantle may be a possible source for the tin and accompanying tungsten and fluorine. A source in the mantle is difficult to prove be-

cause extraction from continental lithosphere, by magmas or magmatic heat provided by rising plume material, cannot be easily discounted. Initial $\text{Sr}^{87}/\text{Sr}^{86}$ ratios of 0.721 and 0.718 determined for samples of granites from complexes in Nigeria²⁹ and Rondônia¹⁸ suggest that significant amounts of the granitic rocks associated with the tin deposits have a crustal source, a hypothesis also supported by other workers²⁶. Much lower initial Sr isotope ratios have, however, been obtained for some granitic units of the White Mountain plume trace in New Hampshire³⁰, possibly indicating a subcrustal origin for their parent magmas. In Nigeria, tin potentially available for extraction, occurs in pegmatites in the Precambrian basement³¹. The abundance of boron, beryllium and tantalum in these deposits and the virtual absence of these elements from the Jurassic tin deposits have been cited as evidence against the hypothesis of remobilisation of Precambrian tin²⁷, unless some mechanism for element segregation is proposed. The rather widespread occurrence of tin in association with anorogenic, alkaline granitic complexes and, perhaps more significantly, its presence along the full length of belts of complexes, also tend to decrease the likelihood of a source in remobilised Precambrian deposits. Furthermore, the absence of significant amounts of the more common base metals from these complexes seems to preclude anatexis or assimilation of unmineralised Precambrian crustal rocks as an origin for the tin. Although some of the granitic rocks are derived at least in part from the continental crust, the evidence, although far from unequivocal, seems to favour a source in the mantle for the tin (compare with ref. 27).

Support for a mantle derivation of lithophile elements at hot spots is provided by evidence from two localities at which continental crust is absent. Blocks dredged from the axial trough of the mid-Atlantic Ridge in the vicinity of the Azores hot spot show evidence of hydrothermal attack and have high contents of several heavy metals, including tin³². At the Iceland hot spot, granitic rocks derived from parent magmas of mantle origin contain concentrations of molybdenum³³, an element which has a geochemical affinity to tin and which occurs with it in some of the complexes (for example, Nigeria⁴ and Sudan¹⁰).

The generation of tin mineralisation above some hot spots and not above others (White Mountain magma series, western Scotland Tertiary province) might reflect either an inhomogeneous distribution of tin (or fluorine) in the mantle or

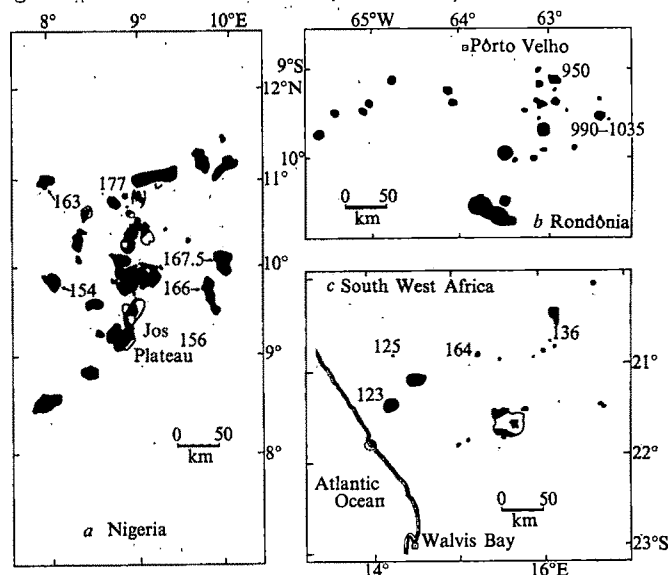


FIG. 2 Linear distribution of tin-bearing, alkaline granitic complexes within elongate belts in a, Nigeria; b, Rondônia; and c, South West Africa. Radiometric ages (numbers) in Nigeria from Jacobson *et al.*²¹ and van Breemen and Bowden¹⁵; in Rondônia from Priem *et al.*¹⁸; and in South West Africa from Siedner and Miller²².

the propagation of mantle plumes, each with a different tin (or fluorine) content, from several levels in the mantle.

The concept of tin mineralisation related to granitic complexes of alkaline type generated in intraplate environments, and unrelated to arc-trench systems, should be useful in the search for ore. In poorly exposed regions, such as Rondônia, the observation that hot-spot activity commonly gives rise to several lines of complexes within an elongated belt (Fig. 2) could facilitate the delimitation of areas for more detailed exploration. Tin deposits might also accompany felsic differentiates at some hot spots in the ocean basins.

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Nature of South Africa's Cape Fold Belt

THE report by Gough¹ of a probable ridge on the highly conductive zone of the upper mantle may be of considerable significance in understanding the deformation of the Palaeozoic trough which became the Cape Fold Belt of South Africa. Until the results of the extended magnetic survey are available, detailed interpretation would be premature, but a few comments may offer guidance for future geophysical work.

The Cape Fold Belt is difficult to explain on plate tectonic models, since (a) it shows a complete lack of any significant igneous or regional metamorphic activity, and (b) it consists of two folded chains almost at right angles, the main one trending E-W, and the smaller one at its western end NNW. These two belts meet at what can best be described as a convergence, since both swing round to a SW direction and merge (Fig. 1).

I have suggested² that an explanation should rather be sought in terms of vertical tectonics and gravity sliding, and a model based on this premise was worked out. The immediately relevant part of this model involved uplift of the southern flank of the main depositional trough (whose axis of maximum subsidence runs just north of the present-day mountain belt) to provide the slope on which gravity sliding took place. It seemed necessary, however, to explain certain zones of more intense folding which occur just north of the major E-W faults, and it was suggested that these faults were active at a very early stage, and with downthrows to the north, forming a series of steps down which gravity sliding took place.

At a later stage, during the breakup of this part of Gondwanaland, the prevailing crustal tension would have induced new movement along these existing lines of weakness but this time with downthrows to the south, as seen today.

The conductive anomaly as shown in Gough's Fig. 1 underlies an area of maximum uplift indicated by the outcropping of late Precambrian basement, on the north side of a major fault line, and it is therefore important to know whether the anomaly is confined to the vicinity of the fault, or whether it extends much further southward (the latter is implied by Gough's statement (ref. 1, page 94) that less than half the anomaly was covered by the array of magnetic stations and by Fig. 9 in ref. 3). It is also important to know whether the same or similar anomalies are associated with other fault lines further south.

Gough suggests that the conductive anomaly may pass eastwards into the gravity anomaly described by Hales and Gough⁴, which is associated with the Cretaceous-filled Algoa basin on the depressed southern side of one of the major

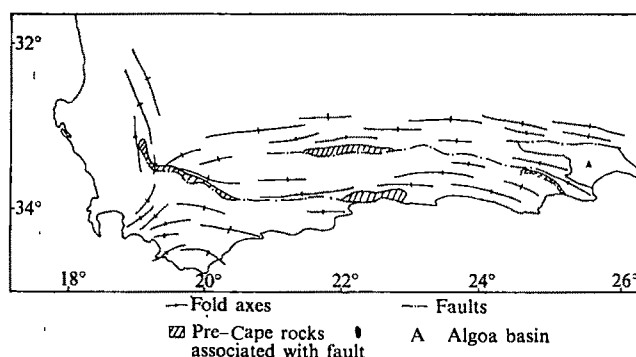


FIG. 1 The Cape Fold Belt.

faults. If, however, there is any close connection between the proposed upward bulge (I prefer this term to 'plume' in this context) on the surface of the highly conductive mantle and the crustal uplift, the conductive anomaly should pass north of the gravity anomaly; it will be interesting to see what the extension of the magnetic survey reveals.

The basic mechanism I invoked for the formation of the Cape Fold Belt was one of vertical movements of fault-defined blocks but no driving force for such movements could be suggested at that time. Clearly, however, if Gough's interpretation of the conductive anomaly is correct, and if such linear mantle bulges can form and decay over long periods, they could provide an adequate explanation for 'keyboard tectonics' in the overlying crust and thus may explain not only the recent uplift of the Cape mountains but also their earlier generative stages.

I thank Dr R. A. Scrutton and C. J. Harnady for discussions.

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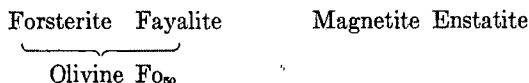
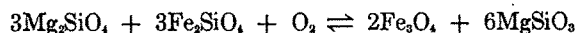
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Oxidation of natural olivines

THE oxidation of olivine is a commonly recognised natural phenomenon and has been investigated experimentally using crystallographic and thermodynamic techniques^{1,2}. It is generally believed that the oxidation of the olivine occurs by initial breakdown of the fayalite component, and subsequent reaction with the forsterite component, to give magnetite and orthopyroxene³. The oxidation of olivine of composition Fo₈₀ can be envisaged as two part reactions:



that is,



In natural olivines such a reaction is commonly represented by partial or complete replacement of the olivine by orthopyroxene-magnetite aggregates. A variation of this reaction is, however, observed in several suites of Precambrian olivine-bearing cumulate rocks which are intrusive into granulite facies gneisses in the western Musgrave Block in central Australia. In such cases a compositional control on the oxidation process is apparent.

(1) In relatively fayalitic olivines (Fo₈₃₋₇₈), olivine is partially or completely altered to granular orthopyroxene-vermicular magnetite aggregates.

(2) In more forsteritic olivines (Fo₇₈₋₈₄), primary spinel inclusions in the olivine are surrounded by thin (0.01 to 0.2 mm) rims of fibrous to granular orthopyroxene. Small quantities of vermicular to granular dark green spinel are commonly intergrown with the orthopyroxene. The spinel core is largest where associated with the thickest rims, suggesting that the primary spinel has grown by the addition

of reaction product spinel. Limited electron microprobe data suggest that both spinel types are of similar picotite composition. Spinel inclusions in other phases are not rimmed.

(3) In the most forsteritic olivines (Fo₈₅₋₈₈), no signs of alteration were observed even though euhedral green spinel inclusions were present.

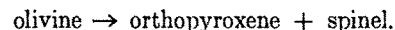
It is believed that the orthopyroxene-magnetite intergrowths in the more fayalitic olivines result from subsolidus oxidation by the type of reaction shown for olivine of composition Fo₈₀.

Such a reaction involves a volume decrease of approximately 3%. Relative volumes of one magnetite to three enstatite would be produced and this is approximately what is observed. The orthopyroxene produced would be much more Mg-rich than the primary olivine as a result of the oxidation of the fayalite molecule. Both optical and electron microprobe examinations support this prediction.

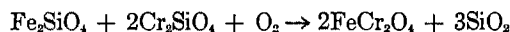
Granular orthopyroxene and opaques, apparently pseudomorphous after olivine, have been reported in granulite facies rocks apparently penetrated by a transient oxidising fluid phase⁴. Very large areas of the Adirondacks granulite facies terrain have undergone oxygen isotope exchange with some pervasive fluids⁵. The cause of oxidation in the central Australian rocks is, however, unknown. The oxidation may be related to the development of interstitial hydrous fluids, because olivines of composition Fo₈₅ in the Kalka Intrusion are only oxidised in rocks containing biotite. Alternatively, reheating during post-consolidation deformation in the area may have increased oxygen fugacity⁶.

The oxidation is relatively late stage. Coronas typical of the subsolidus high pressure olivine-plagioclase reaction (olivine + plagioclase → orthopyroxene + clinopyroxene + spinel) are commonly observed in this area. In some cases these double coronas surround orthopyroxene-opaque aggregates, indicating that oxidation occurred after the olivine-plagioclase reaction.

The orthopyroxene-spinel intergrowths presumably formed by similar oxidation of more magnesian olivines in the presence of spinel:



The spinel is picotite, (Fe, Mg) (Al, Fe, Cr)₂O₄, which corresponds to the magnetite, FeFe₂O₄, in the more iron-rich oxidation reaction. The presence of chromium in at least some olivine lattices is indicated by the chromite⁷ or chrome magnetite exsolution plates in some of the olivines from the area. The chromium was probably originally incorporated into the olivine as Cr²⁺ in isomorphous substitution with Mg²⁺ and Fe²⁺ (ref. 8). Part of the equivalent oxidation reaction for more magnesian olivines could therefore be:



that is, the spinel phase would contain some chromite component.

The origin of the aluminium in the spinel is more difficult to explain. It may have been derived from the associated primary spinel inclusions (as could the chromium) which were subsequently homogenised with respect to the vermicular spinel. The alternative source, Al-bearing phases (for example plagioclase) outside the olivine, involves diffusion problems because movement would need to be through the olivine lattice. It is, however, interesting to note that Muir and Tilley⁹ propose movement of Mg, Fe and Si through olivine lattices during similar oxidation processes in some Kilauean picrite basalts.

The lack of oxidation in the most forsteritic olivines, even in the presence of primary spinel inclusions, indicates the greater stability of the more magnesian olivines under oxidising conditions.

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Stream abrasion of flint implements

Most finds of European Lower Palaeolithic flint artefacts are made from ancient river gravels, either as what are assumed to be concentrations of freshly made implements, suggesting an occupation site, or as abraded material indicating stream redeposition. Verbal description of any abrasion is inaccurate, because lightly abraded specimens may appear fresh to the naked eye. The widths of ridges can be measured on implements with a microscope, thus allowing unworn artefacts and distinct assemblages within a given population to be identified.

When flakes are struck off a flint nodule, either by natural or human agency, the resulting flake scars are separated by a narrow ridge, which is extremely sharp in the fresh state. As the nodule becomes abraded the width of the ridges increases and is easily measurable. The abrasion rate is proportional to the relative hardness of the flint, the velocity of the current, the shape of the nodule or implement, and the particle size distribution of the material present in the transporting medium.

Ridge widths can be measured by placing the artefact under a microscope with incident light from two separate sources converging on the ridge. At least 25 ridges from each

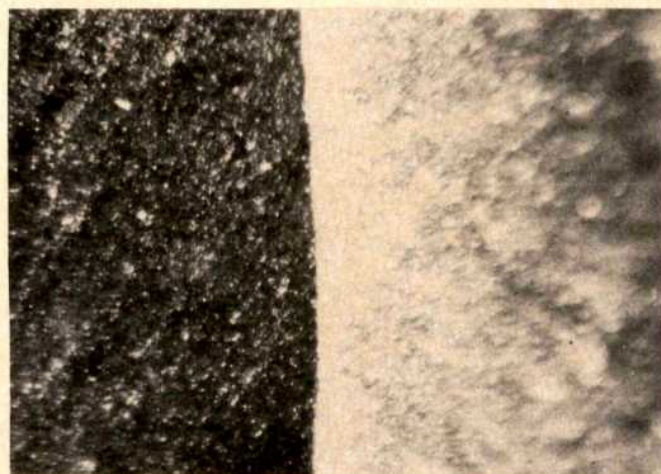


FIG. 1 The fresh ridge. Average width 5 μm . Magnifications of Figs 1-6, $\times 75$.

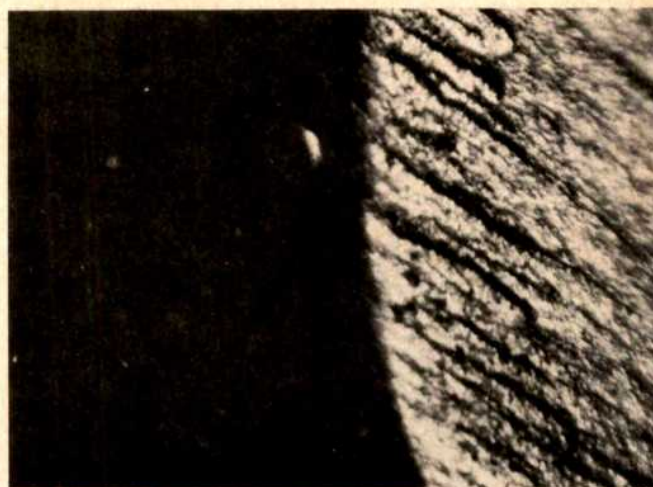


FIG. 2 The appearance of stress cracks. Average width 15 μm .

side of the implement must be measured. Care should be taken to avoid those that have been affected by human use. A suitably calibrated eyepiece and a magnification of $\times 75$ should permit accuracy of $\pm 0.1 \mu\text{m}$. Various chemical coatings, for example silver nitrate, may be used to improve the optical properties of flint for photography, but these tend to obscure the ridges. Direct comparison of the ridge widths of the components of an assemblage is then possible, and may easily distinguish unworn material.

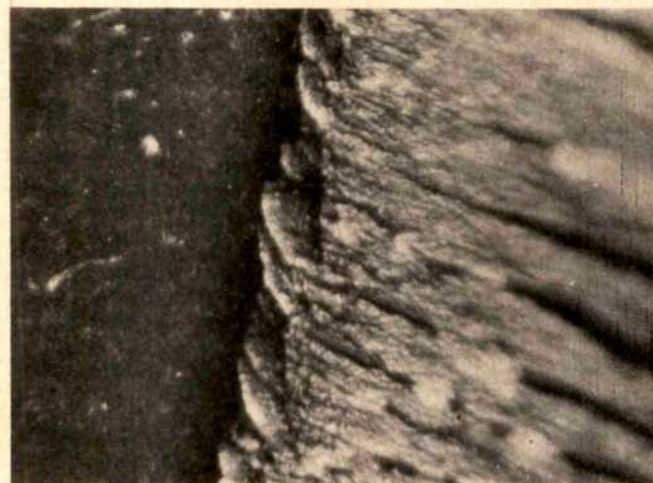


FIG. 3 A 'braided' ridge. Average width 53 μm .

Figures 1-6 show stages in the natural abrasion of a ridge. They illustrate the process and act as standards for the terminology employed. Figure 1 shows a fresh ridge photographed at a magnification of $\times 75$ in incident light. The ridges have average widths of $5 \mu\text{m}$. The abrasion process consists of both chipping of the implement by other natural and humanly worked nodules during transport, and of a slow grinding down of the ridge by an abrasive consisting of the finer elements in the load carried by the stream. The chipping process is much more active soon after the beginning of the abrasion but continues throughout the process at the places nearest to the centre of gravity of the nodule. Initial stages are marked by the appearance of stress cracks (Fig. 2) which develop a 'braided' appearance (Fig. 3). The braiding is caused by pebbles hitting the ridge at an acute angle and striking off microscopic flakes. The braided ridge is then ground away (Fig. 4), causing a gradual increase of ridge width, irrespective of the flaking angle of the ridge. An alternative sequence involves the rapid appearance of percussion craters, caused

TABLE 1 Correlation between observed ridge widths and commonly used verbal descriptions

Observed ridge width (μm)	0-10	10-20	20-50	50-100	100-200	200-300	300+
Common verbal description	Mint condition	Very fresh	Fresh	Slight abrasion	Abraded	Heavily abraded	Very heavily abraded
Suggested index value	0	1	2	3	4	5	6

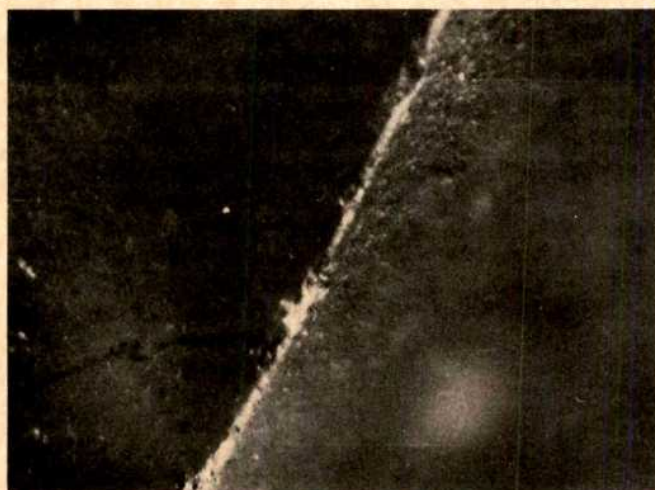


FIG. 4 A 'braided' ridge subjected to grinding in fine clay. Average width 34 μm .

by the impact of a heavy pebble (Fig. 5). The final product is, however, always a smooth ridge crossed by hairline cracks (Fig. 6). The worn ridge may attain a width of 4 mm in heavily abraded material. The nature of the abrasion process is largely a function of the type of abrasive and it is possible to distinguish implements that have been abraded in different environments. If the abrasive is fine grained, such as silt or clay, the relative abrasion is very slow but the rounded appearance of the ridge (Fig. 6) is attained very quickly. Experimental verification of these processes may be obtained by abraiding freshly made implements in a tumbling mill, or sandblaster, with different abrasives and concentrations.

Table 1 shows a correlation between the observed ridge widths and the commonly used verbal description of abra-



FIG. 5 Ridge showing some grinding and percussion craters formed by the impact of large pebbles. Average width 81 μm .

sion. An 'abrasion index' value is suggested, based on these metric criteria, facilitating easy quantification of results.

Using these methods it may be possible to define the depositional environment of stray artefact finds by commenting on the textural character of the abrasive material present in the stream, and to distinguish groupings within apparently homogeneous artefact population. In several cases doubt has been cast on supposedly *in situ* assemblages, which now appear to have been stream rolled. I have recently examined a late Lower Palaeolithic assemblage from river gravels at Great Pan Farm (Newport, Isle of Wight). It was at first



FIG. 6 Heavily abraded ridge showing the final rounded appearance, crossed by fine hairlike cracks. Average width 279 μm .

thought that the implements were manufactured at a nearby occupation site, implying that they were virtually contemporary with the gravel deposit, because they appeared totally unabraded. Closer examination showed that many exhibited clear traces of stream abrasion, and variable abrasion index values. It was therefore concluded that the material represented a random accumulation produced by selective stream redeposition, necessitating a re-examination of the typological characteristics of the false 'assemblage'.

A study of the standard typological sequences using these methods may emphasise the need for caution in interpreting implement associations.

I thank Mr A. M. ApSimon and Dr D. P. S. Peacock for help and criticism. The idea of obtaining experimental verification of the abrasion process was developed on the basis of suggestions and practical help from Peacock.

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BIOLOGICAL SCIENCES

Virus productive transformation of marsupial cells by Schmidt-Ruppin strain of RSV

Rous sarcoma virus (RSV) transforms different species of mammalian cells. The RSV genome is usually not fully expressed in such cells and the virogenic type of virus-cell interaction is obtained^{1,2}. One exception was, however, noted by Svoboda and Klement³ in the case of some hamster tumours induced by Prague strain of RSV (PR RSV) which were found to produce traces (about 1 ID₅₀ per 2.5 g tumour tissue) of infectious RSV.

In order to establish a suitable RSV-producing system in mammalian cells which would be well defined karyologically, we used the marsupial cell line PtK 1 originally derived from the kidney of rat-kangaroo (*Potorous tridactylis apicalis*) by Walen and Brown⁴.

Cells were cultured in medium 199 supplemented with 20% foetal bovine serum. For the transformation, 1.5×10^6 PtK 1 cells were mixed either with 6.4×10^6 resistance-inducing factor (RIF)-free Brown Leghorn chicken fibroblasts (BLEF) infected with Schmidt-Ruppin strain of RSV (SR RSV), or with the same amount of SR RSV-infected BLEF cells which were irradiated with 7,100 R. The mixed cell cultures were designated RSPtK 1 and RS(X)PtK 1, respectively. SR RSV used (SR RSV K18) represents the pure subgroup D virus isolated from a single focus which appeared in the mixed culture of virogenic mammalian cells and chicken fibroblasts⁵. Mixed cultures as well as control PtK 1 cells were subcultured in Roux bottles and split in a ratio of 1:3 at intervals of 3–10 d.

The clone RSPtK 1/K1 was derived from RSPtK 1 cells after 3 month cultivation by picking a single colony of cells containing refractile cells. For colony isolation, a stainless steel cylinder fixed with a silicone grease to the bottom of the dish was used. The plating efficiency of RSPtK 1 cells was 1%. RSPtK 1/K3 clone was isolated by seeding one cell with a micropipette on the feeder layer of X-irradiated (7,000 R) PtK 1 cells prepared on Petri dishes. The growing clone was obtained in one out of ten dishes.

After 4 month cultivation the cell lines were checked for the presence of avian gs antigen using the immunofluorescence method. This antigen was detected in both RSPtK 1 and RS(X)PtK 1 cells.

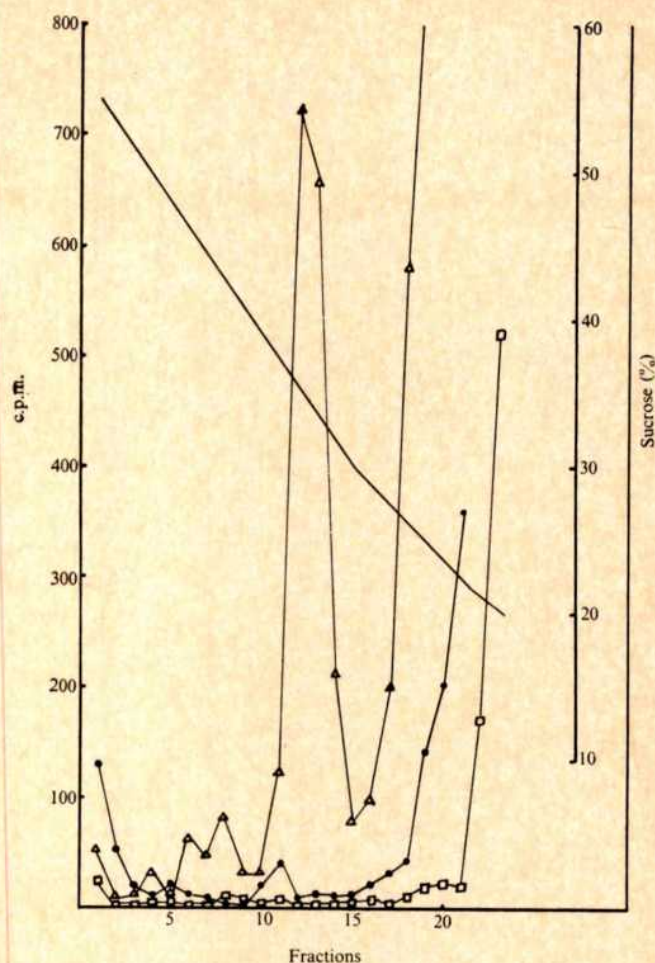


Fig. 1 Culture medium (10 ml) from virus producing RSPtK 1 and control PtK 1 cells labelled for 24 h with $10 \mu\text{Ci ml}^{-1}$ of ^3H -uridine or ^3H -thymidine was clarified by centrifugation at 10,000 r.p.m. for 10 min and layered over 50 ml 10%–58% linear sucrose gradient. The samples were centrifuged in the MSE 3 \times 65 ml rotor in a MSE 65 MkII ultracentrifuge at 20,000 r.p.m. at 6° C for 4 h. Fractions of 2 ml were collected from the bottom and assayed for sucrose concentration and after TCA precipitation for radioactivity. The maximum radioactivity of incorporated ^3H -uridine was found in the peak of density 1.156 which is characteristic of C-type RNA viruses. No radioactivity was found after ^3H -thymidine labelling, as well as in the control cells. Δ — Δ — Δ , ^3H -uridine, RSPtK 1; \square — \square — \square , ^3H -uridine, PtK 1; \square — \square — \square , ^3H -thymidine RSPtK 1.

In addition, all cell lines were tested for the presence of RSV genome and infectious RSV. Living cells from RSPtK

TABLE 1 History and biological properties of PtK 1 cells and RSV-transformed cell lines derived from them

Designation of the cell line	Mode of transformation	Morphology	Generation time (h)	Plating efficiency in soft* agar	Modal chromosome No.	Virus production (FFU ml ⁻¹)
PtK 1	—	Normal	45	<10 ⁻⁶	11	0
RS(X)PtK 1	Co-cultivation with chicken fibroblasts infected with SR RSV, irradiated with 7,000 R	Normal to transformed	34.5	<10 ⁻⁶	11	12
RSPtK 1	Co-cultivation with chicken fibroblasts infected with SR RSV, without X-irradiation	Transformed	20.5	10 ⁻³	11	22 25 (with 100 μg DEAE dextran) 15 (filtrate) 50
RSPtK 1/K1	Clone derived from RSPtK 1 cells	Transformed	NT	10 ⁻⁵	11	50
RSPtK 1/K3	Clone derived from RSPtK 1 cells	Transformed	NT	NT	11	2

* Average number from two dishes



Fig. 2 Morphology of RSPtK 1 cells which shows a non-oriented pattern of growth and presence of stellate and fibroblastoid cells ($\times 55$).

1, RS(X)PtK 1 and RSPtK 1/K1 cells gave rise to Rous sarcomas after injection of relatively small numbers of living cells (about 10^3). Moreover, infectious virus was detected when chickens were injected with cell-free culture fluid collected from full-grown cell layers, either centrifuged at $3,000g$ for 30 min or filtered through Millipore membranes ($0.22 \mu\text{m}$ pore size).

The mean titre of virus as measured by the focus assay method⁶ varied from 1.2×10^4 to 5×10^4 FFU ml^{-1} between different lines with the exception of RSPtK 1/K3 clone kept for a long period of time in tissue culture where RSV production was lower (Table 1).

RSPtK 1 cells were also studied by electron microscopy. Cells were fixed with glutaraldehyde, scraped with a rubber policeman, pelleted, post-fixed with osmium tetroxide and after dehydration the pellets were embedded in Araldite. Ultrathin sections were cut with a Reichert Om U 2 ultramicrotome, contrasted with uranyl acetate and lead citrate and observed with a JEM 7 electron microscope. Early stages of budding of virus particles as well as mature C-type particles were clearly identified.

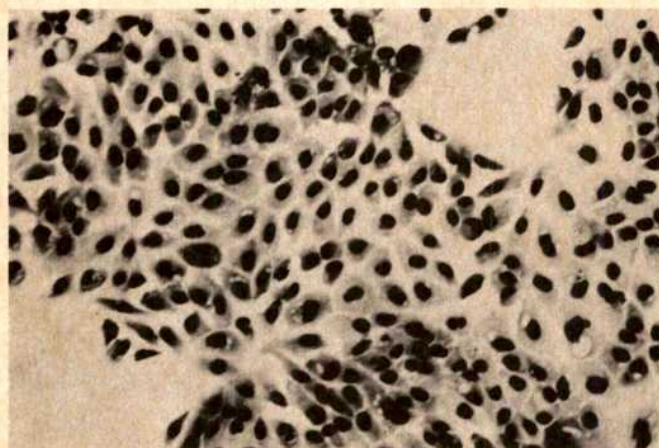


Fig. 3 Morphology of PtK 1 cells. Epithelioid cells with the tendency to form packed colonies ($\times 55$).

If RSPtK 1 cells were grown in the medium containing ^3H -uridine and the supernatant was centrifuged in 10–58% sucrose density gradients a peak of radioactivity at a density of 1.16 g cm^{-3} was detected (Fig. 1). The virus isolated from RSPtK 1/K1 cultures was neutralised by two log dilutions with the specific anti-SR RSV-D antiserum⁵ to the same extent as SR RSV-D virus. Transformed cell lines cultivated

over a year tended to lose their virus-producing capacity and to become virogenic.

Further biological parameters of all cell lines are summarised in Table 1. RSPtK 1 and RSPtK 1/K1 cell lines plated in soft agar⁷ and were morphologically transformed. Figure 2 illustrates the morphology of RSPtK 1 cells which displayed a non-oriented pattern of growth; the cell population consisted of stellate and fibroblastoid cells. The generation time of RSPtK 1 cells was half that of control PtK 1 cells. In contrast to it, PtK 1 and RS(X)PtK 1 cell lines did not form colonies in soft agar and retained the epithelioid morphology with a tendency to form packed colonies on the solid substrate (Fig. 3). But after prolonged passages RS(X)PtK 1 cells also became morphologically transformed.

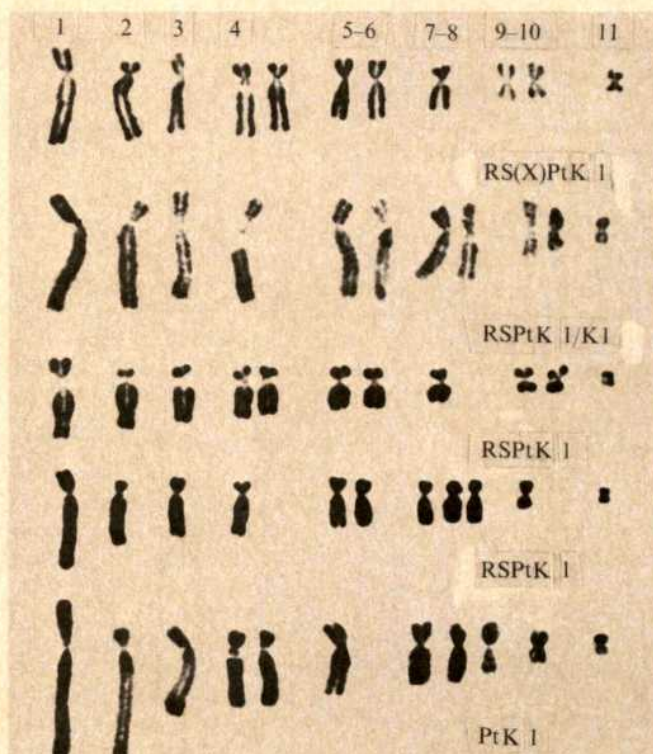


Fig. 4 Karyotypes of PtK 1 cells and RSV-producing cell lines derived from them ($\times 544$).

All cell lines had the eleven chromosome stemlines (Fig. 4) and the frequency of chromosomal damage was not increased above the control background in the RSV-producing lines.

The model of RSV-transformed PtK 1 cells offers a unique possibility for investigating RSV production on a permanent cell line which is well defined karyologically and for studying which host cell chromosomes are necessary for the maintenance of the virus-producing state.

This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research (U.S.A.). We thank Dr J. E. Shannon, American Type Culture Collection, Rockville, Maryland for providing the cell line PtK 1.

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DNA synthesis in 'nuclear monolayers' from BSC-1 cells infected with herpes virus

HERPES simplex viruses (HSV) produce latent (as well as overt) infection in man. Recent evidence seems to indicate that they can also transform mammalian cells in culture^{1,2}. Furthermore, seroepidemiological³ and molecular hybridisation⁴ studies have suggested a possible causal relationship between genital infection with HSV type II and cervical cancer.

A detailed understanding of viral DNA replication is necessary to elucidate the process of HSV infection. Considerable information has been obtained about the changes, caused by infection, in the soluble enzymes associated with DNA synthesis, for example, the induction of a virus-specific DNA polymerase^{5,6} and deoxynucleoside kinase^{7,8}, soon after infection.

In recent years, however, the use of carefully isolated, insoluble replication complexes (or toluenised cells) has transformed our views on the roles of soluble polymerases in the replication of the bacterial chromosome⁹⁻¹¹. It therefore seemed likely that similar insights into the mechanism of herpes DNA replication might be obtained with an analogous mammalian system. Friedman and Mueller¹² have demonstrated the ATP-dependent incorporation of deoxynucleoside triphosphates into the DNA of isolated HeLa nuclei and showed that this activity was correlated with the synthetic capacity of the intact cells. To prepare the active nuclei the cells were disrupted in hypotonic buffer, the cytoplasm was removed by repeated centrifugation and the nuclei suspended in a suitable buffer. The main drawbacks of this

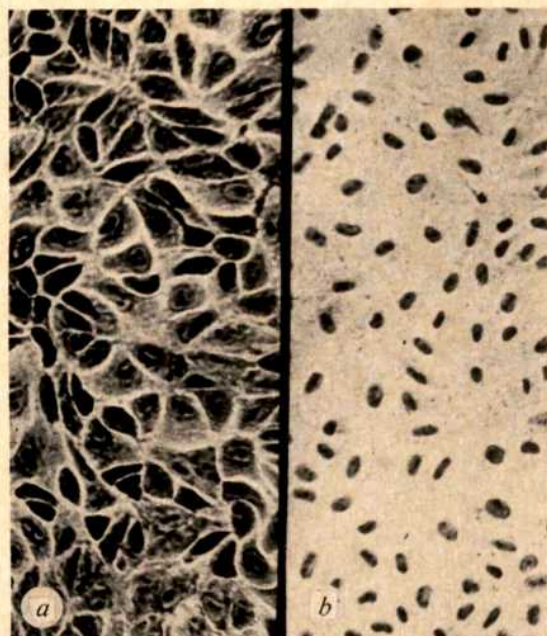


Fig. 1 A confluent monolayer of BSC-1 cells before (a) and after (b) treatment with 0.5% NP40 25 mM HEPES buffer pH 7.6, 1 mM Mg Cl₂ and 0.5 mM CaCl₂.

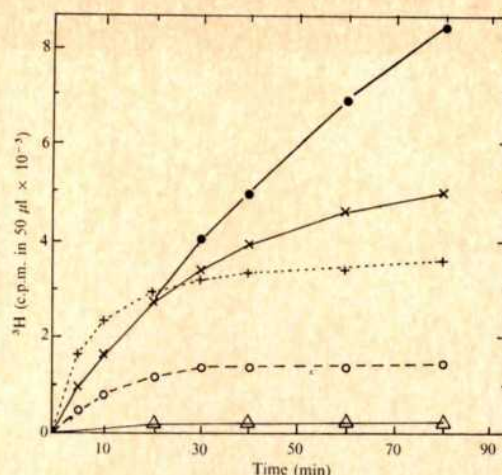


Fig. 2 Kinetics of ³H-dTTP incorporation at 37° C. BSC-1 cells were grown to confluence in Eagle's medium in 5 cm Petri dishes and infected at a multiplicity of infection of 10 with HSV type 1 strain α (2×10^7 P.F.U. per 0.2 ml per dish) or mock-infected with 0.2 ml medium. After 1 h the cell sheets were washed twice with 3 ml medium and overlaid with a further 2 ml. At 6 h after infection the medium was removed and 2 ml of cold 0.5% NP40, 1 mM MgCl₂ and 0.5 mM Hepes buffer, pH 7.6 were added. After a minute or two this was decanted and the resulting 'nuclear monolayers' washed with 1.0 ml of the incorporation buffer minus ³H-dTTP (see text), at various pH values. Complete incorporation buffer (0.5 ml) at the appropriate pH was then added to each dish and the dishes incubated in sealed humidified boxes for 45 min at 37° C. The reaction was stopped by adding 2 ml ice-cold 0.15 M NaCl/0.015 M sodium citrate/0.01 M EDTA. The nuclei were scraped off, transferred to tubes, made 0.5% with respect to sodium dodecyl sulphate and heated at 65° for 10 min. After cooling, pronase (500 μ g ml⁻¹) was added and the mixture incubated for 16 h at 37° C. Samples (50 μ l) were taken and the acid-insoluble radioactivity determined by the paperdisk method of Bollum¹³. \times , pH 7.6; \bullet , pH 7.6 but more dNTPs (25 pmol dATP, dCTP, dGTP and 10 μ Ci ³H-dTTP per dish) added after 20 min; +, pH 7.9; \circ , pH 7.1; Δ , nuclei from mock-infected cells, at pH 7.6.

method are that preparation takes at least 30 min, nuclei can be damaged, or lost, due to clumping or rupture during handling and the nuclear pellet is difficult to re-disperse completely.

This report describes a quick and convenient method of nuclear preparation and the results obtained by its application to the study of DNA replication in BSC-1 cells infected with wild-type (wt) and temperature sensitive (*ts*) mutants of herpes simplex viruses. A brief report of the use of similarly prepared 3T3 cell nuclei in the study of RNA transcription has recently appeared¹³.

If BSC-1 cells are treated *in situ*, with the non-ionic detergent NP40 (0.5% in 25 mM HEPES buffer pH 7.6 containing 1 mM MgCl₂, 0.5 mM CaCl₂ and 1 mM dithiothreitol) the cytoplasm is removed, leaving the nuclei and part of the cell membrane firmly attached to the culture vessel as a 'nuclear monolayer' (Fig. 1). Some shrinkage of the nuclei does occur but few are lost and no whole cells are detectable. The nuclear monolayer is stable and can be washed to remove cytoplasmic contamination. Autoradiographic evidence suggests, however, that some ribosomes remain attached to the residue of the cell membrane which surrounds each nucleus.

When BSC-1 cells which have been infected with herpes simplex viruses are treated in this way 5-9 h after infection (when viral DNA synthesis is most active *in vivo*) the resulting nuclei retain a high capacity for viral DNA synthesis. Under the appropriate conditions (50 mM HEPES buffer, pH 7.6, containing 10 mM MgCl₂, 100 mM NaCl, 0.5 mM CaCl₂, 1 mM dithiothreitol, 50 μ M dATP, dCTP and dGTP, 20 μ Ci ml⁻¹ ³H-dTTP and 5 mM ATP, see

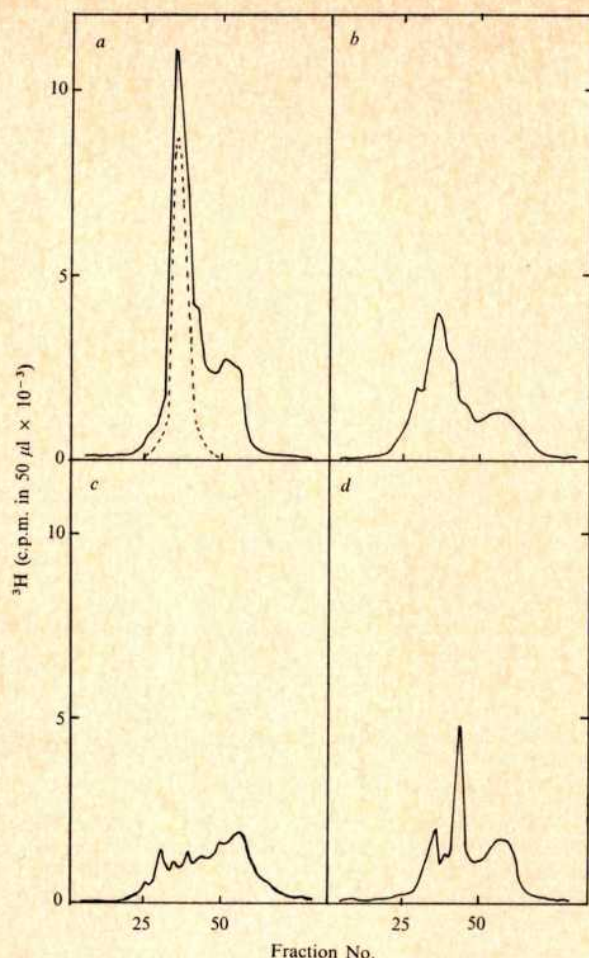


Fig. 3 Pycnographic analysis of DNA labelled *in vitro* with ^3H -dTTP in modified incorporation mixtures. *a*, Complete incorporation mixture as described in the text; *b*, complete mixture minus ATP; *c*, complete mixture minus NaCl; *d*, complete mixture but with ATP, CTP, GTP and UTP at $30\ \mu\text{M}$. Incorporation conditions and DNA analysis as described in Figs 2 and 6. The position of virion DNA (....) is also shown in (*a*).

Fig. 2) extensive synthesis occurs. Up to 100 pmol (around 100,000 c.p.m.) can be incorporated per 10^6 nuclei in 60 min at 37°C , and the initial rate of synthesis, during the first 5 min can be as high as $10\ \text{pmol min}^{-1}$. These figures, which are minimum estimates based on the incorporation of added ^3H -dTTP (making no allowance for any endogenous dTTP) compare very favourably with recently published studies in which the techniques of Friedman and Mueller were used to examine the replication of DNA in nuclei from adenovirus KB cells infected with adenovirus type 5^{14,15} and 3T6 cells infected with polyoma¹⁶. Although incorporation falls off fairly rapidly, the addition of more deoxyribonucleoside triphosphates (dNTPs) at 20 min maintains the existing rate for almost another hour (Fig. 2), indicating that a major cause of the reduction in the rate of synthesis is probably lack of substrate; due presumably to degradation. This has not been checked directly.

The addition of more ATP at this time has no effect. This is perhaps to be expected as the ATP concentration in the mixture (5 mM) is already fairly high. Although ATP is required for full activity, a substantial, but variable, amount of incorporation (10–65% of that in the complete mixture) occurs even in its absence. It is not clear whether this is due to residual endogenous ATP or to a DNA synthetic activity which does not require ATP.

If magnesium, or all four dNTPs, are omitted, however, synthesis is reduced to less than 5%. Similarly, if NaCl is not added to the incorporation buffer, synthesis, particularly

of viral DNA (see Fig. 3), is severely inhibited. Dithiothreitol, (the omission of which has little effect) is added as a precautionary measure since the sulphhydryl antagonist N-ethylmaleimide (at 20 mM) completely inhibits DNA synthesis in these nuclear preparations.

Under these conditions nuclei prepared from mock-infected cultures exhibit almost no DNA synthesis (Fig. 2). Since these cells are subject to contact inhibition of growth and movement, and have been confluent for at least 2 d before use, there is little *in vivo* DNA synthesis compared with a logarithmically growing population. The activity detected *in vitro* is, however, considerably lower than would be expected. This may mean that the residual DNA synthesis in resting cells depends on a soluble polymerase which is lost during preparation of the nuclei.

As expected, nuclei from virus-infected cells will incorporate radioactivity from any of the four dNTPs. It is, however, surprising to find that ^3H -TdR is also readily incorporated, provided the other three dNTPs are supplied (see Fig. 4). No incorporation occurs in the absence of ATP, nor can any of the other deoxyribonucleosides be incorporated. This activity is not therefore due to a few whole cells which have survived the detergent treatment. Since these nuclei are washed before assay, it seems that this activity is bound either in, or to, the nucleus, or alternatively, to the remains of the cell membrane.

This observation is of particular interest since it is well established that one consequence of herpes virus infection is the appearance of a virus-specific thymidine kinase^{7,8} shortly before the onset of viral DNA synthesis. In mock-infected cells very little ^3H -TdR is incorporated.

A direct comparison of the absolute amounts of DNA synthesised *in vivo* and *in vitro* in a given time cannot be made without greater knowledge of the many parameters involved (such as pool sizes and rates of synthesis). It is not known whether initiation of new rounds of DNA synthesis occurs *in vitro* or whether those molecules already in the process of replication when the nuclei are prepared are merely completed. Nevertheless, when the DNA synthesised *in vitro* is banded on a CsCl gradient the relative amounts of viral and cellular DNA always reflect the *in vivo* pattern throughout the viral growth cycle (see Fig. 5). Closer examination of the CsCl profiles, however, reveals that the *in vitro* labelled viral DNA appears to consist of several species with slightly different densities, rather than a single homogeneous peak. This heterogeneity (a trace of which can often be seen even in viral DNA prepared from whole cells) is more noticeable

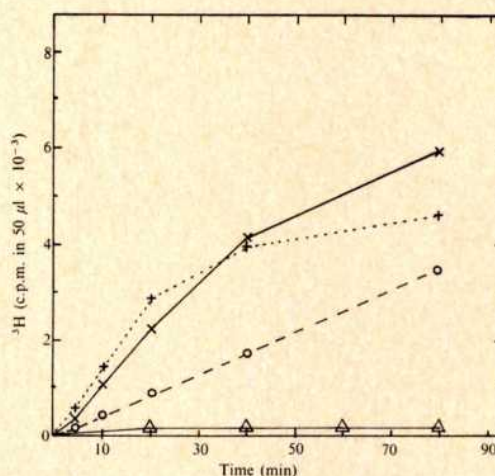


Fig. 4 Kinetics of ^3H -TdR incorporation at 37°C . Cells were infected and nuclei prepared as in Fig. 2. The incorporation protocol was also the same, except that ^3H -TdR ($20\ \mu\text{Ci ml}^{-1}$) replaced dTTP in the incorporation mixture. \times , pH 7.6; \circ , pH 7.1; $+$, pH 7.9; Δ , nuclei from mock-infected cells at pH 7.6.

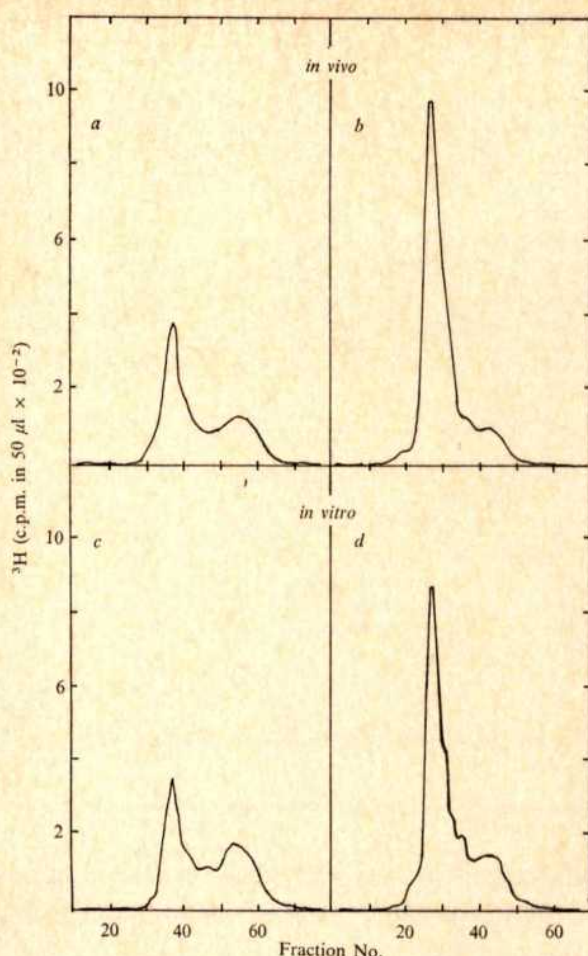


Fig. 5 Pycnographic analysis of DNA labelled *in vivo* (a and b) with ^3H -TdR, or *in vitro* (c and d) with ^3H -dTTP. At 3 h (a) and 7 h (b) after infection, cells were exposed to ^3H -TdR ($5 \mu\text{Ci ml}^{-1}$ in Eagle's medium) for 45 min at 37°C . The medium was decanted, the cell sheet washed with 3 ml phosphate buffered saline and cytoplasm removed by treatment with 0.5% NP40. The nuclei were scraped off and DNA extracted as described in Fig. 2. Nuclear monolayers were also prepared 3 h (c) and 7 h (d) after infection, labelled with ^3H -dTTP ($20 \mu\text{Ci ml}^{-1}$) for 45 min and DNA extracted as before. ^{32}P -labelled phage T4 DNA was added to the DNA thus obtained and the solutions adjusted to a density of 1.723 g ml^{-3} with CsCl and centrifuged in a Spinco Ti 50 rotor for 3 d at 40,000 r.p.m. Five-drop fractions were collected and the acid-insoluble radioactivity to $50 \mu\text{l}$ samples determined. The bottom of the tube is on the left and viral DNA bands at the denser position.

on some occasions than on others. It is accentuated by omissions from, or additions to, the incorporation mixture (Fig. 3). The various components (probably five in number) seem to occur at fairly consistent densities but in varying amounts and, so far, remain unexplained. Some have buoyant densities greater than virion DNA and others have densities intermediate between virion and cellular DNA. It has not yet been determined which peaks are viral, and which cellular, in origin. They may arise from both sources and exhibit greater buoyant densities due to the presence of RNA sequences and/or some degree of single strandedness. Attempts to distinguish between these possibilities have been unsuccessful. The main 'viral' DNA peak is most affected by the omissions; the host peak much less so. At this stage, however, it is still not clear whether these components are intermediates in viral DNA synthesis or merely artefacts.

DNA synthesis was studied in nuclear monolayers prepared from cells infected with *ts* mutants of HSV type II. Mutants of HSV type I were not used because it was difficult to obtain good titres in these cells. (This problem has now been

largely overcome.) The type II mutants (isolated by Timbury¹⁷ and characterised by Halliburton and Timbury¹⁸ in this Institute) were therefore used, although these have certain drawbacks. The switch off of host cell DNA synthesis is poor, the viral eclipse phase tends to be protracted at the permissive temperature (31°C) and the wt virus itself is slightly inhibited at the non-permissive temperature (38°C).

Cells were infected with either of two mutants of HSV II; *ts5*, a DNA-positive mutant (in which viral DNA synthesis appears to be unimpaired at 38°C) and *ts1*, a DNA-negative mutant (in which no viral DNA is made at 38°C). After 7 h at 38°C , when DNA synthesis in cells infected with *ts5* was known to be proceeding rapidly, both batches of cells were cooled to 31°C and *in vivo* DNA synthesis was measured by a 45 min pulse of ^3H -TdR. At the same time nuclear monolayers were prepared and exposed to ^3H -dTTP for 45 min also at 31°C . The labelled DNA was isolated and banded on a CsCl gradient (Fig. 6). The absolute amounts of radioactivity *in vivo* and *in vitro* cannot of course be compared directly, but the *in vitro* profile mirrors the *in vivo* picture in both cases. Thus, in *ts5* infected cells, where viral DNA synthesis appears to be proceeding normally, it can be detected *in vitro*. In the case of *ts1*, on the other hand, no viral DNA synthesis can be seen, *in vivo* or *in vitro*, in the 45 min following the change to the permissive temperature. This probably means that under these conditions a step essential for the assembly of the synthetic apparatus and initiation of DNA synthesis is blocked and that there has been insufficient time at the permissive temperature for the production of detectable amounts of viral DNA. In contrast to this is the finding (J. Hay, personal communication) that both *ts5* and *ts1* when grown at the non-permissive temperature produce normal (or greater than normal) amounts of the soluble herpes-specific DNA polymerase assayable in extracts of infected cells.

It is, therefore, of particular interest to know whether, in the case of *ts1*, the DNA synthetic apparatus, once it has been established at the permissive temperature, still exhibits temperature sensitivity and whether this is demonstrable *in vitro*.

Cells were infected with *ts1* and maintained at 31°C for 16 h (when the rate of viral DNA synthesis is highest in this

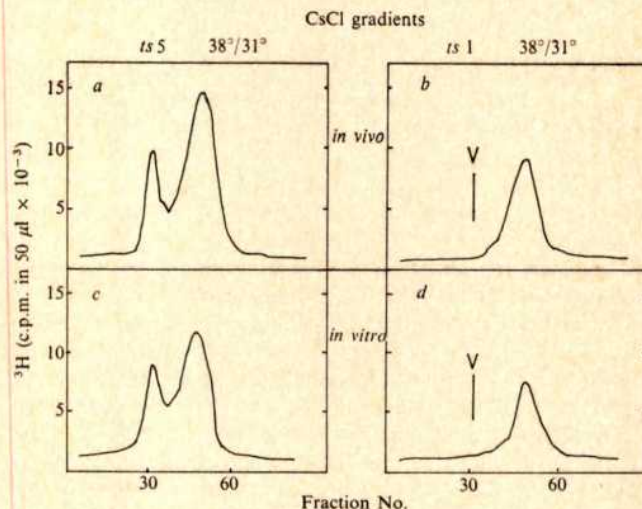


Fig. 6 Pycnographic analysis of the DNA produced *in vivo* (a, b) and *in vitro* (c, d) by *ts* mutants of HSV type II. Cells were infected as described in Fig. 2 with either the DNA-positive mutant *ts5*, or the DNA-negative mutant *ts1*. After absorption, the temperature was raised to 38°C until 7 h after infection when the cells were cooled to 31°C and either labelled for 45 min *in vivo* with (a, b) ^3H -TdR ($5 \mu\text{Ci ml}^{-1}$ in Eagle's medium) or converted to nuclear monolayers and labelled for the same time with (c, d) ^3H -dTTP, as described in Fig. 2. The DNA was extracted and banded as described in Fig. 5.

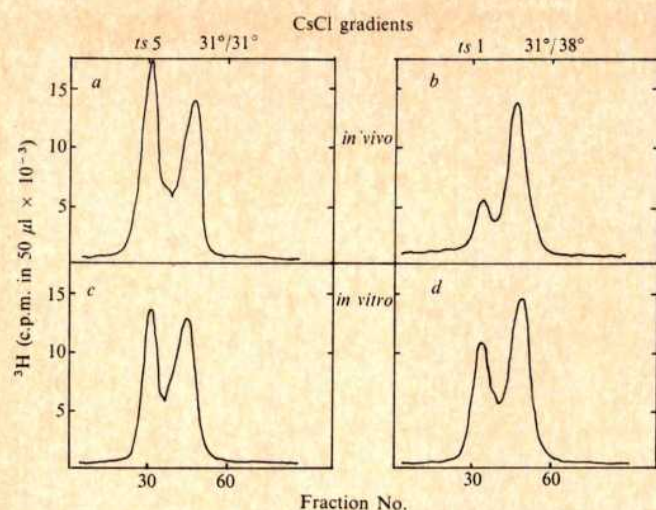


FIG. 7 Pycnographic analysis of the DNA produced *in vivo* (a, b) and *in vitro* (c, d) by HSV II *ts1* at the permissive and non-permissive temperatures. Cells were infected as described in Fig. 2 with the DNA negative mutant *ts1*. At the end of absorption the temperature was lowered to 31° C until 16 h after infection when whole cells, and nuclear monolayers were labelled for 45 min at 31° C, or raised to 38° C for 10 min and then labelled for 45 min at that temperature. The DNA was extracted and banded as in Fig. 5.

mutant). Whole cells and nuclei were then assayed, at both 31° C and 38° C, for incorporation of ^3H -TdR or ^3H -dTTP respectively. (Fig. 7). The viral DNA synthetic apparatus appears to be quite clearly temperature sensitive *in vivo*. The effect *in vitro*, although always in the same direction, is much less marked. This may well mean that the *ts* function contributes less to the overall rate of DNA synthesis *in vitro* than *in vivo*. These experiments are always difficult to control accurately, because of factors such as variation in the synthetic rates at different temperatures, the effect of temperature on the timing of the viral cycle and differences in pool sizes, such that the changes *in vitro* are to some extent masked by the inherent variability of the system. So, although this technique probably gives rise to nuclei which are capable of temperature-sensitive DNA synthesis *in vitro*, further experiments involving a variety of *ts* mutants will be required to confirm this. In addition several important questions remain unanswered. What is the exact nature of the DNA product? What is the role of cytoplasmic factors? Can reinitiation occur?

Nevertheless, these results establish that to a large extent the DNA synthesis observed *in vitro*, in this system, represents true, although perhaps incomplete, viral DNA replication. This method, which enables the quick and gentle exposure of the site of DNA synthesis in a form accessible to precursors, drugs and enzymes, when used with the many *ts* mutants of HSV types I and II now available, can contribute greatly to the elucidation of the molecular biology of herpes virus replication and the problems of mammalian DNA synthesis.

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Multigenic control of ribosomal properties associated with cycloheximide sensitivity in *Neurospora crassa*

It has been shown by McKeehan and Hardesty¹ that, during protein synthesis by 80S ribosomes, cycloheximide selectively inhibits movement of peptidyl-tRNA from the acceptor to the donor site, and that the coupled hydrolysis of GTP, catalysed by the soluble factor T-II in combination with

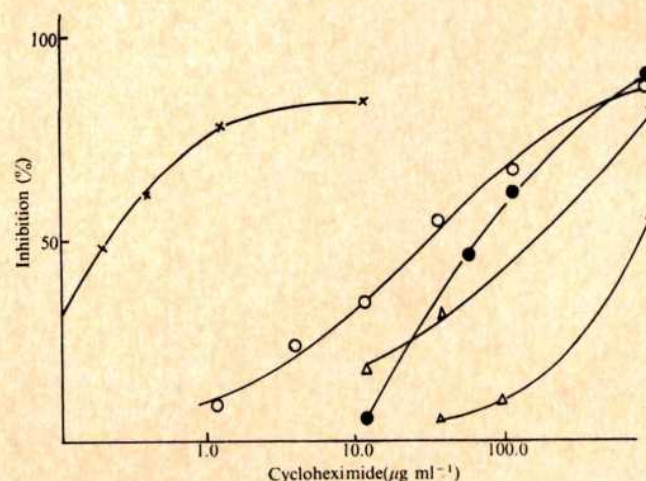


FIG. 1 Effect of cycloheximide on the poly(U)-directed ^{14}C -phenylalanine incorporation into protein by cell free preparation of wild-type and cycloheximide-resistant mutants of *Neurospora crassa*. Protein synthesis *in vitro* was studied in a mixture containing: 50 mM Tris-HCl, pH 7.8; 2 mM ATP; 1 mM GTP; 2 mM phosphoenol pyruvic acid; 15 μg pyruvate kinase; 20 mM KCl; 10 mM mercaptoethanol; 10 mM magnesium acetate; 50 nCi ^{14}C -phenylalanine, specific activity 400 mCi mmole⁻¹; 20 μg poly(U); ribosomes equivalent to 20 μg rRNA and 75 μg of S-100 protein, in a volume of 0.25 ml. Ribosomes washed once in the extraction buffer were used. \times , Wild type, STA; \circ , CH94; Δ , CH40, two different experiments; \bullet , CH96.

TABLE 1 Effect of cycloheximide on the wild-type STA strain of *Neurospora crassa* and on three mutants isolated as resistant to the inhibitor

Strains	ID ₅₀ (μ g cycloheximide per ml of medium)*			Resistance on agar (μ g cycloheximide per ml of medium)†	Locus mutated
	growth (dry weight)	Protein synthesis <i>in vivo</i>	Protein synthesis <i>in vitro</i>		
CH40	20.0	15.0	100.0–1000.0	400.0	<i>act-2</i>
CH94	9.0	10.0	25.0	200.0	<i>act-1</i>
CH96	12.0	10.0	50.0	200.0	<i>act-5</i>
STA	0.2	0.3	0.3	1.0	none (wild type)

* The 50% inhibitory dose.

† Threshold concentrations of cycloheximide for inhibition of colony formation on solid medium.

the ribosomes, is not affected. They proposed that cycloheximide might inhibit protein synthesis by occupying sites on the ribosomes usually available for peptidyl-tRNA. As direct association of the T-II catalysed reaction with translocation has not so far been demonstrated, and GTP hydrolysis by T-II is not inhibited by cycloheximide, it is probable that sensitivity to cycloheximide is entirely associated with ribosomes. This was first proposed by Siegel and Sisler² on the basis of species specific differences in the sensitivity to cycloheximide in yeasts and later supported by the work of Wilkie and his associates³. In the latter case a number of cycloheximide resistant mutants have been studied *in vitro*; one of these mutants, having a low level of resistance, seemed to have altered ribosomes.

Resistance to cycloheximide at a high level can result from mutation in any one of at least four genes in *Neurospora crassa*. These genes have different alleles determining different levels of resistance. They are also subject to modification by independent genes⁴. I have investigated three resistant strains, CH94, CH40 and CH96, carrying single gene mutations at the *act-1*, *act-2* and *act-5* loci, respectively, to determine their levels of resistance and the subcellular function which has been modified in each case.

Rapidly collected middle-log phase mycelium, grown from conidia in a New Brunswick Fermentor at 30° C in Vogel's medium⁵, supplemented with 0.1% yeast extract and 2% sucrose, was used for the preparation of the cell free extracts. Preparations of ribosomes and S-100 fractions, as well as assays of the transfer reaction in polyphenylalanine synthesis *in vitro*, were performed essentially as described by Nirenberg⁶. Components of the reaction mixture are indicated in the legend to Fig. 1. Following 10 min incubation at 34° C, 150,000 c.p.m. were detected in hot trichloroacetic acid (TCA)-insoluble material when ribosomes equivalent to 1 mg rRNA were present in the reaction mixture. Dry weight increase in liquid shake cultures during 10 h at 30° C in the presence and absence of cycloheximide, was measured and the % inhibition calculated. Erlenmeyer flasks containing Vogel's medium supplemented with 2% sucrose were inoculated with conidia.

The effect of cycloheximide on protein synthesis *in vivo* was studied in 1 ml samples of 10 h old shake cultures, grown as described above, each incubated at 34° C for 10 min with 20 μ Ci ¹⁴C-phenylalanine, specific activity 10 μ Ci μ mol⁻¹, and cycloheximide. Radioactivity incorporated into protein was measured by the rapid assay system developed by Nirenberg⁶. Sensitivity of growth on solid medium was scored by eye after 2 d incubation at 30° C. Plates, containing Vogel's medium supplemented with 0.3% sucrose, 1% sorbose, and solidified with 2% agar, were spot-inoculated with mycelium.

It is shown in Figs 1 and 2 that in *N. crassa*, mutations in any one of at least three different genes considerably decrease sensitivity to cycloheximide in cell-free extracts, indicating that these genes control components of the protein synthesis apparatus. The effect of cycloheximide on

'hybrid' systems with ribosomes from wild type and supernatant from mutant, or *vice versa*, depends only on the sensitivity of the ribosomal fraction. Thus, the three genes studied in this work control ribosomal components. These mutations are easily scored on agar media because at least a 200-fold increase of cycloheximide concentration is required to inhibit colony formation of the resistant strains in comparison with wild type. Scoring of resistance of growth and *in vivo* protein synthesis in liquid cultures is also easy (Table 1).

It is interesting to notice that in the results obtained in this work the three genes cannot be easily differentiated *in vivo* by level of resistance, in spite of the fact that the decreased sensitivity of all three resistant mutants is clear

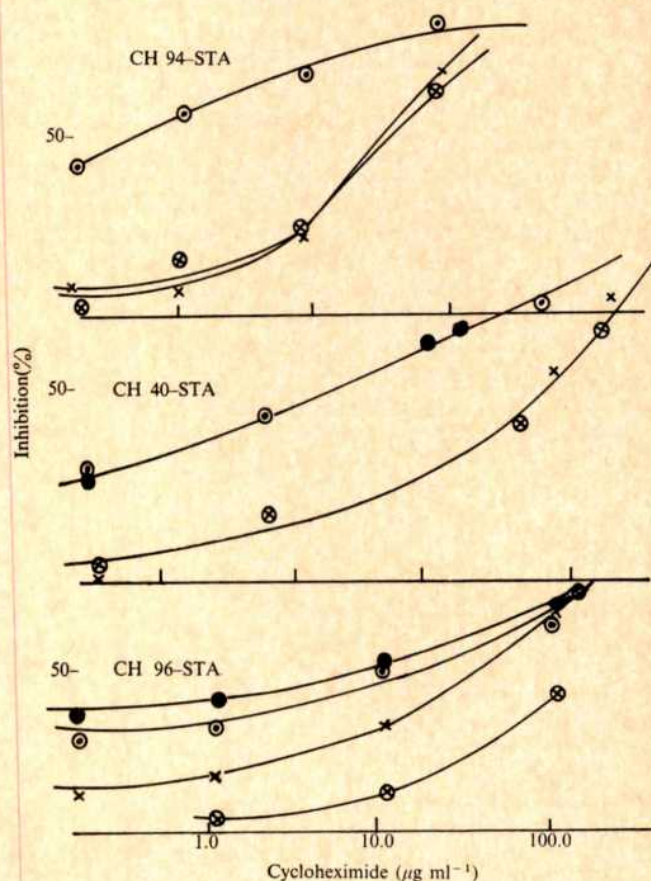


Fig. 2 Response to cycloheximide of cell-free systems of ribosomes and supernatant from wild type and three cycloheximide resistant mutants of *Neurospora crassa*. Protein synthesis *in vitro* was assayed in the mixture described in the legend to Fig. 1, except that ribosomes, washed once in the extraction buffer adjusted to 0.5 M NH_4Cl were used. ●, Wild type system; ×, resistant system; ○, hybrid system with ribosomes of the wild type; ⊕, hybrid system with ribosomes of the resistant type.

cut. *In vitro*, the resistant mutants are even less sensitive than *in vivo* and three different levels of resistance can be recognised, though the *in vitro* resistance of strain CH40 is variable.

The genetic control of resistance of cycloheximide in *N. crassa* was first studied by Hsu⁷. Two unlinked genes, *act-1* and *act-2*, located between well defined markers, have been identified. Hsu has shown that both *act-1* and *act-2* are dominant in heterokaryons and that the double-resistant homokaryotic recombinants obtained from *act-1* × *act-2* are characterised by extremely slow growth rate and almost complete insensitivity to cycloheximide.

My work shows that three genes, two of which are those studied by Hsu⁷, control ribosomal properties related to sensitivity to cycloheximide. It is not yet known whether rRNA or ribosomal proteins are the components modified by the three mutations or whether the genes concerned are structural genes for these components or specify modifying factors as in the case of resistance to kasugamycin in *Escherichia coli*⁸. The dominance of mutations at the *act-1* and *act-2* genes, demonstrated by Hsu⁷, is easily compatible with the idea that these genes specify diffusible factors able to transform all ribosomes in heterokaryons to resistance. On the other hand, if these genes control the primary structure of ribosomal components, their occurrence at scattered sites in the genome is against the idea of more than one of them controlling rRNA. Whatever the modified component may be, however, the extremely slow growth rate of the double resistant (*act-1*, *act-2*) homokaryotic recombinant, observed by Hsu⁷, indicates that some essential function other than binding of the antibiotic is supplied by the normal products of these genes, which is only weakly present in the mutants. That this function is translocation is an interesting possibility.

This work was carried out at the Department of Genetics, University of Leeds, while I held a Scholarship from the Greek State Scholarship Foundation. I wish to thank Professor J. R. S. Fincham for his helpful advice, criticism and support during the course of this investigation and also for critically reading this manuscript.

While this paper was being prepared for publication, Pongratz and Klingmüller reported (*Molec. gen. Genet.*, **124**, 359; 1973) that in *N. crassa*, cycloheximide resistant mutants carrying mutations at the genes *act-1* and *act-2* are resistant *in vitro*.

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Depression of polymorph counts by various scrapie agents

It has been reported by Carp, Licursi, Merz and Merz^{1,2} that the percentage of circulating polymorphonuclear neutrophils (PMN) in C57Bl mice was reduced permanently following injection with either multiple sclerosis or scrapie tissues. They reported that the decrease was first detected 3 d after injection in the case of scrapie and that the decrease persisted throughout the preclinical phase of the disease: most of their results were from mice bled 6 weeks after injection. We report here the confirmation of some of their findings and also discuss certain difficulties which can arise with this test.

Two inbred strains of mice C57Bl, VM and their F₁ cross were used in one mouse colony (ABRO) and C57Bl in another colony (MRI) in various experiments extending in all over 9 months. In one type several biologically different strains of mouse-passaged scrapie³ were used, in a second sheep and goat scrapie (natural or experimental cases) were used, while in a third an attempt was made to confirm the presence of the PMN factor at titres higher than scrapie infectivity and evidence for its replication was sought. In another series of experiments 22A mouse-passaged scrapie was injected into C57Bl, VM and C57Bl × VM mice as a time sequence study from 42 to 158 d. In all experiments etherised mice were bled brachially for PMN counting 6 weeks after injection, or later in the time sequence study.

In all cases intracerebral injections of male mice were used: with the exception of the titrations, inocula were 10⁻² saline brain homogenates (500g, 10 min supernatant). Brains of the appropriate genotype from normal animals or ones with other neurological diseases were used as control inocula. Because it can be much more difficult to recognise different types of leukocytes in mice than in many other species, especially the recognition of PMN with 'tight' nuclei, all the slides were coded and differential counting done 'blind' by Mrs P. Licursi who had made the original observations.

With six different agents tested in ABRO C57Bl mice (22C, 79A, 87A, 51C, 125A and 139A) the PMN percentages were each significantly lower than in the controls ($P < 0.01$) but with 79V agent tested in VM mice the reduction was not significant. Further work is necessary before the important conclusion could be reached that 79V is not associated with any PMN factor. In the time sequence study with 22A agent there was a reduction in each of the three genotypes of mice at 42, 70, 106, 136 and 158 d and this was significant ($P < 0.02$) in 10 of the 15 groups but there was no PMN change in the three groups at 89 d. With six sources of scrapie from sheep (138A, 141A, 161M, RLE, SSBP/1/21, 161P) and two from goats ('scratching' and 'drowsy' types) there were also significant PMN reductions in MRI C57Bl mice compared with the controls which included a pool of 20 brains from normal sheep and sheep with the Border disease or swayback. In the above work mice from both colonies were used and the C57Bl PMN values in control mice ranged from 12.5 ± 0.45 to $16.8 \pm 0.75\%$ whereas the values in the experimental groups ranged from 4.9 ± 0.79 to $10.6 \pm 0.53\%$. Later work was restricted to C57Bl mice in the MRI colony when normal or ME7-scrapie C57Bl brain was used at dilutions down to 10⁻⁸. These attempts to check whether the PMN factor had a higher titre than scrapie infectivity and whether it replicates, failed because the control mice on this occasion had much lower PMN values (5.7–8.8%) than on the previous occasions and were similar to the scrapie-injected groups (5.2–7.7%). Subsequent checks of PMN counts in the MRI colony in uninjected C57Bl mice also showed similar low values (6.6 to 8.9%).

There are two major features in these results. The general findings confirm and extend to numerous strains of scrapie the report of a factor in scrapie-brain which reduces the

circulating PMN values in mice. There seem to be other unidentified factors, however, which can depress the number of circulating polymorphs for considerable periods of time within a colony. It is probably important that in our earlier group of successful experiments the control C57Bl PMN values were very similar to those reported originally by Licursi *et al.*² It may therefore be necessary for this test to use, as controls, C57Bl mice which have average PMN counts in excess of 12%.

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Can energy generated by sugar efflux be used for ATP synthesis in *Chlorella*?

CELLS of *Chlorella vulgaris* can be induced to accumulate hexose analogues more than one thousand fold^{1,2}. This active uptake can be driven by respiration or photosynthesis^{1,3}. During the steady state of 6-deoxyglucose accumulation, (when sugar influx equals sugar efflux) in the dark with air, only about half the influx is powered by respiratory energy and half the steady-state flux rates are still observed under nitrogen^{3,4}. This part of the steady state flux, can, however, be completely inhibited by uncoupling agents such as dinitrophenol or FCCP (ref. 3). It has been postulated, therefore, that about half of the 6-deoxyglucose steady-state influx is supplied with energy generated by efflux.

But can this form of energy, 'efflux energy', only be used for transport into the cell or can other energy requirements of the cells be met by it? For example, the carrier-mediated efflux of ions (Ca^{2+} or K^+) can generate ATP—apparently by the reversal of the energy-dependent influx reaction⁵⁻¹⁷.

To answer this question for *Chlorella* the following experiment was designed. The uptake of glucose and its assimilation is limited by energy under anaerobic conditions in the dark; much higher rates are observed under aerobiosis. When the fate of radioactive glucose in such cells is followed and compared with that of cells preloaded with non-radioactive 6-deoxyglucose, it should be possible to decide if the efflux energy, generated by 6-deoxyglucose leaving the cells, can be used for the ATP-requiring processes of glucose assimilation. *Chlorella* cells were preloaded with unlabelled 6-deoxyglucose until an inside concentration of 0.1 M was reached. These cells were centrifuged and washed with buffer to remove 6-deoxyglucose. Radioactive glucose was added to these cells in anaerobic conditions and the uptake and metabolism of glucose was followed. These data were compared with data obtained with anaerobic cells, not preloaded with 6-deoxyglucose (Fig. 1). Cells not preloaded show a low rate of uptake for glucose of 34 μmol per h per ml packed cells (PC). The glucose taken up is rapidly metabolised; predominantly to glucose phosphate, sucrose and ethanol-insoluble products, which are almost exclusively starch. Hardly any free glucose can be detected.

Cells which were preloaded with 6-deoxyglucose showed a higher rate of uptake (278 μmol per h per ml PC). The rate

of phosphorylation, however, as well as the rate of sucrose and starch synthesis was very similar to that of control cells. Free glucose was considerably accumulated, therefore, and the amount of glucose present after 4 min was clearly higher than the theoretical concentration equilibrium. It has to be emphasised that in no other uptake condition was it possible to observe that *Chlorella* contains a significant amount of free glucose.

The amount of ATP which is required for glucose assimilation is known to be 1 ATP per glucose phosphate, 3 ATP per sucrose and 2 ATP per each glucose incorporated into starch. The ATP utilised for glucose assimilation has been calculated for both experimental conditions and it was found to be identical for non-preloaded cells (51.5 μmol per h per ml PC) and for cells preloaded with 6-deoxyglucose (53.5 μmol h⁻¹ ml⁻¹ PC).

The energy derived from 6-deoxyglucose efflux is quite capable therefore, of driving glucose influx which is increased by a factor 8.18, but it is apparently not able to sustain glucose metabolism by synthesising ATP.

The possibility that 6-deoxyglucose present in the cells prevents the metabolism of glucose, by competitive inhibition for example, has at least been excluded for the hexokinase reaction by *in vitro* experiments.

The results reported here can be explained by the proton symport theory of Mitchell⁹. According to this theory, the proton-translocator movement should be reversible in principle, obeying only the concentration of protons on each side of the membrane. When cells have accumulated high amounts of sugar and the steady state (influx equals efflux) has been reached, protons should also move outward, cotransported with sugar against the proton gradient. In this way sugar efflux would partly restore energy for sugar influx. In addition, any proton gradient, including that created by efflux, could theoretically be transformed to ATP, when a proton dependent ATPase is part of the membrane across which the proton gradient exists. As no additional ATP synthesis has

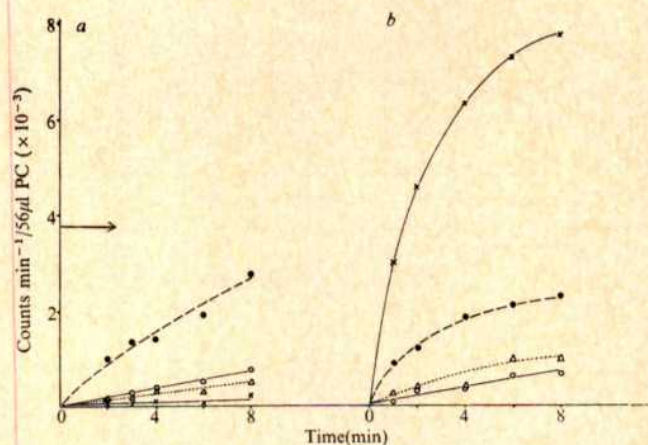


Fig. 1 Uptake and metabolism of ^{14}C -D-glucose by, a, *C. vulgaris* and, b, by *C. vulgaris* preloaded with 0.1 M 6-deoxyglucose in anaerobic conditions in the dark. Packed cells (PC) 300 μl of induced algae¹, were incubated anaerobically³ in 12 ml 0.025 M sodium phosphate buffer, pH 6.5. The uptake experiment was started by addition of 0.8 μCi ^{14}C -glucose at a final concentration of 5×10^{-3} M. Samples of 2 ml were withdrawn filtered and the cells were extracted in 80% ethanol. The radioactive compounds were separated by paper chromatography and the amounts determined as described previously⁸. The algae used for experiment a did not have detectable amounts of free hexoses inside, whereas the algae for experiment b had been incubated aerobically for several hours in 1×10^{-2} M non-radioactive 6-deoxyglucose and were then centrifuged to remove external 6-deoxyglucose. All experiments were performed in the dark at 27°C. ●, Glucose phosphate; ○, sucrose; △, insoluble; ×, glucose; →, concentration equilibrium.

been observed, however, this indicates that either no such ATPase exists or that it is not able to compete with the uptake systems for protons. A third explanation might be that the energy available is inadequate.

That a proton gradient might really be the driving force for sugar transport in *Chlorella vulgaris* is indicated by recent results. Experiments with energy poisons suggest that a high-energy intermediate of respiration formed before ATP is the relevant metabolite for active hexose transport in *Chlorella*¹⁰. In addition, it has been possible to observe pH change corresponding to a proton symport, when transportable sugars were added to induced *C. vulgaris* cells¹¹.

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Implications of rigescent integuments as a new structural feature of some algal chloroplasts

CURRENT concepts concerning the origin of plastids have revived the symbiotic theory of Mereschkowski¹ and Faminzin². Evidence in support of this theory has come from recent work on *in vitro* culture of chloroplasts³⁻⁵. We provide further evidence here by the demonstration of a rigescent integument surrounding the chloroplasts of some siphonaceous algae.

During the preparation of chloroplasts from *Caulerpa sedoides*³, we noted that, in distilled water, there was no swelling or bursting of the plastids as might have been expected. It proved very difficult to break them by any of several methods; they withstood blending in a Waring blender, homogenising in a Ten Broek grinder, freezing and thawing, and sonication. The only method to give quantitative disintegration of the chloroplasts was the French pressure cell used at 8,000 pounds inch⁻².

Detergents such as 0.1% Triton X-100 and Tween 80 had no observable effect on the morphology of the chloroplasts and 1% cetyltrimethylammonium bromide (CTAB) removed chlorophyll only after 48 h and several changes of solution. Sodium dodecyl sulphate (SDS) at 1%, removed chlorophyll and internal lamellae rapidly but left an intact rigescent integument inside which was the intact starch sheath around the pyrenoid (Fig. 1). This integument or 'ghost' withstood boiling in distilled water and centrifugation up to 8000g without any observable change occurring in its structure. It also resisted the action of both pronase and lysozyme.

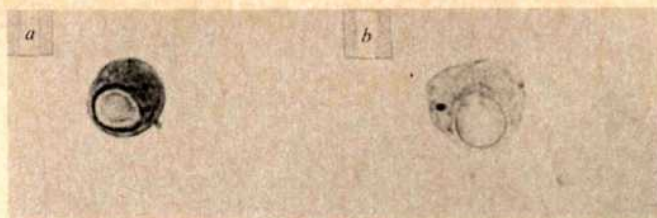


FIG. 1a. Chloroplast of *Caulerpa sedoides* isolated and resuspended in distilled water. $\times 484.50$ Under phase contrast microscopy. b, A rigescent integument prepared from the chloroplasts of *Caulerpa sedoides* by treatment with 1% SDS. The 'ghost' is without chlorophyll and lamellae but retains a characteristic 'butterfly' shape and an apparently unaltered starch sheath around the pyrenoid. $\times 48450$ Stained with toluidine blue under phase contrast microscopy.

The ghosts were weakly birefringent when examined by polarising microscopy, but scanning electron microscopy showed that they lacked any marked surface features and appeared relatively smooth (Fig. 2). In a related species, *C. cactoides*, transmission electron microscopy and interference contrast microscopy of SDS-treated plastid integuments showed that the terminal body of the plastid, considered by some^{6,7} to be a part of the membranous lamellar system of the chloroplast, appeared to be a coherent and integral part of this integument, and remained attached to it even after treatment with SDS. The role of this terminal structure can be inferred from its behaviour during plastid division, where, early in the formation of the characteristic 'butterfly'-shaped chloroplast, the body seems to divide or duplicate and is thereafter carried in the tip of each 'wing'. This suggests that it is in some way involved in the maintenance of polarity in the chloroplast, which at this stage shows remarkable symmetry and alignment of its lamellar system (Fig. 3). A similar, though smaller and more diffuse terminal body, has been found in the plastids of *C. sedoides*, but its behaviour during division has not yet been followed satisfactorily.

The chloroplast integument has been demonstrated in several species of *Caulerpa* (*C. sedoides*, *cactoides*, and *simpli-cuiscula*) and raises the problem of its possible function. Many saccoglossan opisthobranchs eat species of siphonaceous algae almost exclusively and the primitive forms seem to prefer species of *Caulerpa*⁷. It seems possible that the rigescent integument around the chloroplasts of some of these

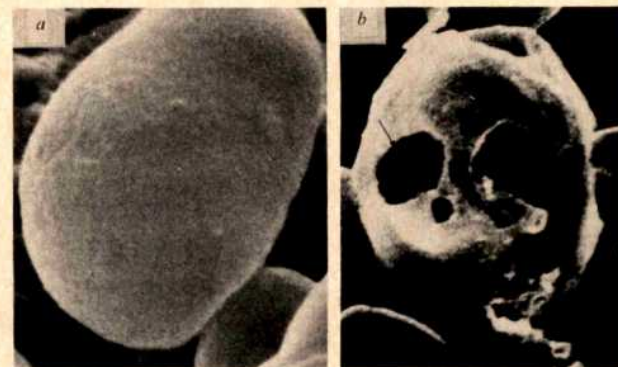


FIG. 2 Exterior surface of rigescent integument of *Caulerpa sedoides* chloroplast as seen with the scanning electron microscope. The surface appears smooth. $\times 5,700$. b, A rigescent integument of *Caulerpa sedoides* chloroplast which was broken during preparation for scanning electron microscopy to reveal not only the starch sheath around the pyrenoid inside the integument (arrowed) but also the 'ghost-like' nature of the integument. The small bodies adjacent to the integument are amyloplasts. $\times 5,700$.



Fig. 3a. An *in vivo* plastid of *Caulerpa cactoides* apparently before division, with the terminal swellings of the integument positioned at the tips of the 'butterfly wings' creating two axes of polarity in the lamellae. $\times 207,480$. b, A chloroplast of *Caulerpa cactoides* showing the remarkable symmetry of lamellae just before division. $\times 2,109$.

species protects them from digestion in the gastric juices of the animal and may be of some selective value in ensuring endosymbiosis in animal tissue.

The discovery of these integuments, however, has a bearing on the endosymbiotic theory of the origin of chloroplasts. Not only do these plastids have an integument which endows them with physical resistance to external conditions, they are also capable of repeated division outside the cell^{4,5}, and have photosynthetic pigments closely related to those of normal green algae and higher plants⁸. The resistance of the integument to the action of lysozyme shows a significant difference from the cell wall material characteristic of the blue-green algae, which have been considered the prime contenders for the role of the primitive chloroplast ancestors. It seems that the plastids of some siphonaceous algae may represent either a missing link in the development of plastids from the ancestral algal symbiont, or an alternative line of plastid evolution.

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Molecular conformation of deoxyguanosine 5'-phosphate

THE allowed conformations of nucleic acids depend on the flexibility of the structure of the nucleotides from which they are constructed. Many X-ray crystallographic studies of the degree of flexibility of these structures have shown that nucleosides exhibit a larger number of preferred conformations than nucleotides, and this led Sundaralingam¹

to suggest that a nucleotide is more 'rigid' than a nucleoside. Berthod and Pullman², however, have drawn attention to the fact that considerations of conformational energy do not imply such a rigid structure.

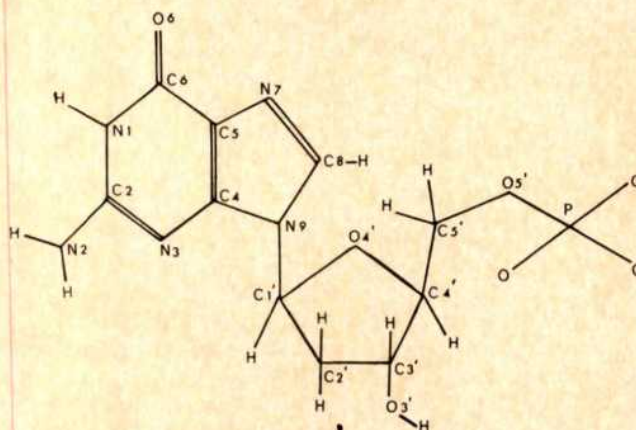
We have recently determined the crystal structure of the disodium salt of deoxyguanosine 5'-phosphate tetrahydrate (GDRP), and we find that the pucker of the sugar ring and also the conformation about the C(4')-C(5') bond are different from those found in other 5'-nucleotides which are normal components of DNA and RNA. These results indicate that a nucleotide may not be as rigid as previous crystallographic studies have implied.

Crystals of GDRP were obtained by evaporation from aqueous solutions. The unit cell is monoclinic with $a = 5.56$, $b = 10.76$, $c = 15.77$ Å, $\beta = 97.9^\circ$, and the space group is P2₁. The structure was solved by Patterson interpretation methods. The positions of the phosphorus atom and one of the sodium atoms were determined from the Harker section, and the orientation of the phosphate group was found by means of a rotation function. Successive Fourier syntheses revealed the positions of the remaining atoms. Details of the structure determination will be given elsewhere. The R factor at the present stage of refinement is 0.06.

The conformation of GDRP about the glycosidic bond C(1')-N(9) is *anti*, with the torsion angle O(4')-C(1')-N(9)-C(4) of 237° . In having the *anti* conformation, GDRP is similar to all other nucleotides in the crystalline state and similar to the majority of nucleosides.

The pucker of the sugar ring is unusual in that relative to the mean plane through the five atoms of the ring, atom O(4') has maximum deviation of 0.25 Å *endo* and atom C(4') has the next largest deviation of 0.21 Å *exo*. This type of pucker has not previously been observed in nucleotides, although it has been observed in the nucleoside dihydrothymidine³.

The conformation about the C(4')-C(5') bond in GDRP is also unusual in that the orientation of the C(5')-O(5') bond relative to the C(4')-O(4') and C(4')-C(3') bonds is *gauche-trans*, with the torsion angle O(5')-C(5')-C(4')-O(4') = 62.6° and O(5')-C(5')-C(4')-C(3') = 175.4° . In all other 5'-nucleotides which are normal components of DNA and RNA, and also in all the refined double-helical models of DNA and RNA, the conformation is *gauche-gauche*. The only nucleotide reported with a *gauche-trans* conformation is 6-azauridine 5'-phosphate (ref. 4), which is not a normal nucleic acid component. Amongst nucleosides, however, the *gauche-trans*, as well as *trans-gauche*, conformations occur quite frequently, but the fact that they had not been observed in nucleotides and dinucleotides was one of the reasons why Sundaralingam suggested that a nucleotide is more rigid than a nucleoside.



The chemical structure of deoxyguanosine 5'-phosphate.

The conformational parameters of GDRP suggest that, depending on the environment, conformations other than *gauche-gauche* are possible about the C(4')-C(5') bond. Indeed, in the study of the binding of nucleotides to lactate dehydrogenase⁵ and to staphylococcal nuclease⁶, the *gauche-trans* conformation is required in order to explain the difference-Fourier maps, and a similar conformation is proposed for a dinucleotide phosphonate when it binds to RNase-S (ref. 7). The present results may also make possible models of single-stranded viral nucleic acids with conformations other than *gauche-gauche*. Finally, it is worth noting that the original Watson-Crick model of DNA⁸ had a *gauche-trans* conformation about the C(4')-C(5') bond, although it was a feature which required modification to *gauche-gauche* in order to fit the X-ray diffraction data⁹.

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Rosette formation by a mouse fibroblast cell line

THE properties and activities of mammalian cell membranes have become an area of increasing interest in cell biology, and their study has involved a variety of experimental approaches. The mutual interaction of membranes from identical or different cell types has often been studied. For example, it has been found that a large proportion of human peripheral blood lymphocytes, when mixed with sheep red blood cells, form cell clusters known as rosettes, in which a central lymphocyte is partially or fully surrounded by red cells¹⁻³. This also occurs with human thymocytes⁴, and is apparently a property of human lymphocytes derived from the thymus (T cells)^{5,6}.

During the course of experiments designed to study the presence of receptors for antigen-antibody complexes on tumour cells⁷, it was found that the mouse fibroblast cell line, A9, made rosettes to sheep erythrocytes which were not coated with antibody. Here we present experiments describing this phenomenon in A9 cells and in cell hybrids between A9 and TLX/5 tumour cells.

Various cultured cell lines were used in this study: (1) A9, a line which lacks guanylic acid-inosinic acid pyrophosphorylase and was originally isolated by Littlefield as a clone of mouse L fibroblasts resistant to 3 μ g ml⁻¹ of 8-azaguanine⁸. (2) TLX/5, which was derived from a mouse lymphoma⁹ and grown *in vitro*. (3) Three hybrid clones,

resulting from the Sendai virus-induced fusion of A9 and TLX/5 cells *in vitro*. The cell fusion procedure used was essentially that of Davidson¹⁰. The hybrid nature of these cells was confirmed by karyotype analysis. (4) Normal mouse fibroblasts, obtained from secondary cultures of newborn mouse brain. (5) Human skin fibroblasts; (6) A3 hamster fibroblasts obtained from cell lines grown *in vitro* for many generations. The cells used were grown on Falcon plastic Petri dishes in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum and 100 IU ml⁻¹ of penicillin, and incubated at 37° C in a humidified atmosphere of 10% CO₂ in air¹¹. Cells were collected from the plates with a rubber policeman and washed twice in gelatin veronal buffered saline (GVB)¹², before being made up to 20 \times 10⁶ cells ml⁻¹ in GVB (other media such as TC199 have been used with similar results).

Red blood cells were obtained from guinea pig, hamster, horse, human, mouse, rabbit, rat and sheep, usually by collecting whole blood in citrated GVB and washing at least three times. 100 μ l of packed red blood cells were added to 10 ml of GVB, which gave a count of approximately 200 \times 10⁶ erythrocytes ml⁻¹ (this was found to be optimum for rosette formation).

0.05 ml (1 \times 10⁶ cells) of the cultured cell suspensions were added to 1 ml of red blood cells in a round-bottomed 5 ml plastic test tube. The cells were mixed and spun down immediately at 150g for 7 min, after which they were resuspended by gently rocking to and fro. 0.33 ml of 2.5% glutaraldehyde in PBS was added to each tube and carefully mixed up to fix the cells¹³. One drop of this suspension was dropped on to a clean slide and allowed to dry. After fixation in methanol, the slides were stained in Leishman's stain and the dried preparations were read¹⁴. In reading the slides, three classes of cells were distinguished; full rosettes were cells which were completely surrounded by erythrocytes, partial rosettes, cells having three or more erythrocytes but were not full rosettes, and non-rosette-forming cells which had no erythrocytes, or one or two, adhering to their surface. Results are expressed as the percentage of these three types.

Table 1 shows the rosette formation of A9 cells with various species of erythrocytes. It can be seen that A9 cells form many rosettes with horse, sheep and hamster, fewer with rat and hardly any at all with the other species tested.

As horse erythrocytes seemed to give more full rosettes than either of the other species tested, we decided to see if any of the other cell lines made rosettes with them. Table 2 shows the results. It can be seen that the only cells which formed any appreciable number of rosettes with horse erythrocytes were A9 and Hybrid C. Two of the hybrids were negative as was the other parental type (TLX/5) and the other cell types tested. This experiment has been repeated using sheep erythrocytes instead of horse erythrocytes with essentially the same results.

The A9 rosettes are sensitive to previous incubation with 0.1% trypsin, or heat killing (56° C, 15 min), but relatively

TABLE 1 Rosetting capacity of A9 cells with various species of erythrocyte

Species of erythrocyte	% Full rosettes	% Partial rosettes	% Non-rosettes	% Full + partial
Horse	46	23	31	69
Sheep	24	53	23	77
Hamster	17	61	22	78
Rat	8	33	59	41
Rabbit	0	10	90	10
Mouse	0	8	92	8
Guinea pig	0	8	92	8
Human	0	4	96	4

TABLE 2 Rosetting capacity of various cell lines with horse erythrocytes

Cell line	% Full rosettes	% Partial rosettes	% Non-rosettes	% Full + partial
A9	39	27	34	66
TLX/5	0	0	100	0
Hybrid A	0	3	97	3
Hybrid B	1	5	94	6
Hybrid C	19	29	52	48
Human skin fibroblasts	0	0	100	0
Mouse brain fibroblasts	0	6	94	6
A3 hamster fibroblasts	0	0	100	0

insensitive to previous incubation with high concentrations of sodium azide.

Many questions are posed by this work. First, since there is a large overlap of cells binding sheep, horse and hamster erythrocytes, is the receptor on the A9 cells responsible for this binding a single receptor, or are there different receptors for sheep, horse and hamster erythrocytes on the same cell?

Second, although A9 cells form rosettes whereas TLX/5 cells do not, only one out of the three hybrid cell lines formed rosettes. One explanation could be that some A9 cells never express the receptor and these were the cells that fused with TLX/5 to form the two negative hybrids. This seems unlikely since the A9 cell line was isolated as a single clone in 1963, and has been cloned several times since then. Another explanation could be that the spatial arrangement of this receptor on the cell surface is critical for red cell attachment. Yet another explanation is that the gene controlling receptor production in A9 was lost during or after fusion with TLX/5, and a corollary to this is that there might be a gene which suppresses receptor production in TLX/5, and that it was this gene which was lost, thus allowing at least one hybrid to show rosette formation. Further observations on the karyotypes of these hybrids should yield information on the possibility of any chromosome loss. The explanations posed above assume that these rosette-forming cells have specific or fairly specific receptors on their surface. The possibility does exist however that rosette formation may be explained by a non-specific phenomenon relating to the physiological conditions of the cells at the time of collection.

Although the A9 cells and one hybrid are the only cells shown here capable of forming rosettes, it might be possible to find other surface markers that are characteristic of certain cell types, such as the lymphocyte in man which binds sheep erythrocytes¹⁻³; this has been suggested as a marker for thymus-derived cells⁵⁻⁶, and is now used routinely in the assessment of T cell function in man¹⁵.

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Antibody coated erythrocytes as a manifold probe for antigens

A variety of antigens can be coupled to erythrocytes and these antigen coated erythrocytes (Ag-E) are widely used for the detection of the corresponding antibody¹. In principle, if erythrocytes were coated with antibody, these antibody-coated erythrocytes (Ab-E) would be useful for the detection of the corresponding antigen. Ag-E and Ab-E should exhibit parallel reversed immunological phenomena, for example, whereas Ag-E are agglutinated and lysed by antibody, Ab-E should be agglutinated and lysed by antigen; whereas Ag-E

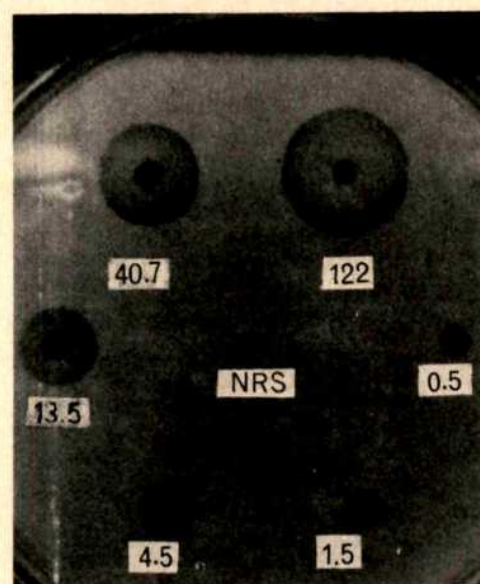


FIG. 1. Disks of haemolysis produced by human immunoglobulin diffusing into gel containing anti-human immunoglobulin Ab-E. Eleven milliliter of 1% Ab-E and 0.8% agarose in saline borate buffer were allowed to gel in a 10 cm Falcon Petri dish. Five μ l of human IgG diluted in normal rabbit serum (NRS) absorbed with sheep erythrocytes were placed in punched wells. The dish was kept at 4° C for 48 h, then incubated with 4 ml of 1:100 dilution of rabbit anti-human Ig antiserum at 4° C overnight and finally treated with 4 ml of 1:10 dilution of guinea pig complement at 37° C for 2 h. The concentrations (μ g/ml) of human immunoglobulin in the wells are indicated in the photograph.

TABLE 1 Immunoglobulin allotype (antigen)-induced agglutination of sheep erythrocytes coated with antibody specific for rabbit kappa chain allotypes

Indicator cells	Antigen	Antigen dilution (\log_{10})								
		-1	-2	-3	-4	-5	-6	-7	-8	-9
Anti-b4 Ab-E	b4 serum	-	-	+	+	+	+	+	+	-
	b5 serum	-	-	-	-	-	-	-	-	-
	b4b5 serum	-	-	+	+	+	+	+	±	-
	b4 IgG (10 mg ml ⁻¹) ₄	-	-	+	+	+	+	+	+	-
	b4 serum + anti-b4*	ND	ND	ND	ND	-	-	-	-	-
Anti-b5 Ab-E	b4 serum	-	-	-	-	-	-	-	-	-
	b5 serum	-	-	+	+	+	+	+	+	-

The antigens were absorbed with sheep erythrocytes and serially diluted with 0.2% gelatin in 0.15 M NaCl. Then, 0.1 ml of the dilutions was incubated with 0.1 ml of 1% indicator cells (Ab-E) in 0.15 M NaCl at 4° C overnight.

* 0.1 ml of the dilution of b4 serum was incubated with 5 μ l of anti-b4 antiserum at 37° C for 30 min before incubating with the Ab-E.

are antibody reactive, Ab-E should be antigen reactive. To test this prediction, we attached various antibodies, purified from antisera by using immunosorbents², to sheep erythrocytes by the chromium chloride method³. These Ab-E were tested with the corresponding antigens by agglutination, haemolysis and immunocytoadhesion methods.

Erythrocytes were coated with the following purified antibodies: goat anti-rabbit immunoglobulin, goat anti-rabbit α_2 -macroglobulin, rabbit anti-bovine serum albumin (BSA), rabbit anti-mouse immunoglobulin, rabbit anti-human immunoglobulin and rabbit antibodies to various rabbit immunoglobulin allotypes (b4, b5, a1, a2 or a3). These Ab-E were incubated with serial dilutions of the corresponding antigens. In all instances, the antigen agglutinated the erythrocytes coated with the antibody specific for the tested antigen. For example (Table 1), in the system with the rabbit b4 immunoglobulin as antigen and anti-b4 Ab-E as indicator cells, rabbit serum or purified IgG having the b4 antigenic determinants agglutinated anti-b4 Ab-E, whereas b5 serum did not. Similarly, b5 serum, but not b4 serum, agglutinated anti-b5 Ab-E. Moreover, anti-b4 antiserum inhibited the agglutination of anti-b4 Ab-E induced by b4 molecules. This antigen-induced agglutination of Ab-E was characterised by a prozone in antigen excess and by end-points at antigen concentrations as low as 1 ng ml⁻¹ or lower (Table 1). In addition, b4 IgG-E and anti-b4 Ab-E co-agglutinated when incubated together, whereas no agglutination was obtained when b5 IgG-E were incubated with anti-b4 Ab-E (not shown).

Ab-E were first treated with the corresponding antigen, then with antibody specific for the bound antigen and finally with complement. The reaction with the corresponding antigen made the Ab-E sensitive to lysis. Thus, anti-b4 Ab-E lysed when sequentially treated with b4 IgG, anti-b4

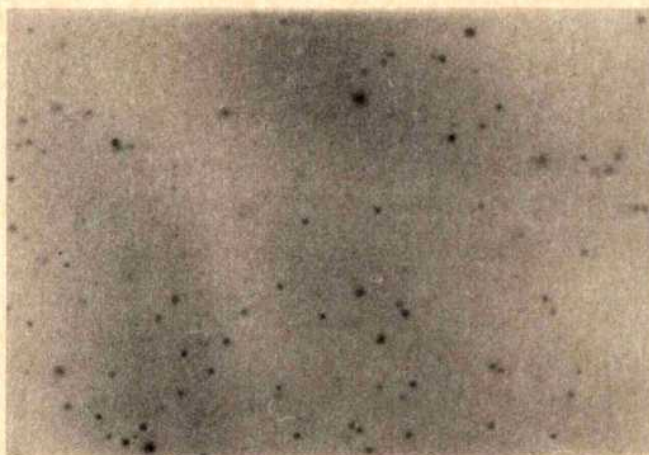


Fig. 2 Haemolytic plaques formed by human lymphocytes with antihuman immunoglobulin Ab-E. Lymphocytes, isolated from blood of a healthy individual by the Ficoll method⁸ were washed with Hanks solution and tested with anti-human immunoglobulin Ab-E by the PFC assay⁵. After incubation at 37° C for 1 h, the dishes were treated with rabbit anti-human immunoglobulin antiserum and guinea pig complement as described in Fig. 1.

antiserum and complement (Table 2). The lysis was specific.

A useful application of this antigen-mediated lysis of Ab-E is the quantitation of antigen by single radial immune haemolysis in gel. When human immunoglobulin at known concentrations was allowed to diffuse from wells into agar gel containing anti-human immunoglobulin Ab-E, localised haemolysis developed around the wells upon addition of rabbit anti-human immunoglobulin antiserum and complement (Fig. 1). At low antigen concentrations, the area of these haemolytic circles, after 48 h, was proportional to the concentration of the antigen. This single radial immunohaemolysis method is a modification of Mancini's method⁴, the major difference being that the antibody is immobilised in the gel by anchorage to the E and the major advantage being that the sensitivity (0.1 μ g ml⁻¹) is greater.

The most important practical use of the antigen-mediated lysis of Ab-E is the detection and enumeration of antigen-secreting cells by an unprecedented PFC (plaque forming cell) assay. In all other PFC assays an antigen either native⁵ or artificial⁶ on the membrane of the erythrocyte is the target for the antibody secreting cells. If Ag-E can detect antibody-secreting cells, Ab-E should detect antigen-secreting cells. More specifically, whereas erythrocytes coated with BSA are an indicator for anti-BSA antibody-secreting cells, anti-BSA Ab-E should be an indicator for BSA-secreting cells. We have indeed found that normal human peripheral lymphocytes formed haemolytic plaques when assayed with anti-human immunoglobulin Ab-E (Fig. 2). Being based

TABLE 2 Immunoglobulin allotype (antigen)-mediated lysis of sheep erythrocytes coated with antibody specific for b4 light chain allotype

Indicator Cells	Antigen	Developing Antiserum	Lysis
anti-b4-E	b4 serum	anti-b4	+
	b4 serum	—	—
	b4 IgG	anti-b4	+
	b4 serum	anti-b9	—
	b9 serum	anti-b4	—
uncoated erythrocytes	b4 serum	anti-b4	—

Two ml of 1% Ab-E and 0.8% agarose in 0.15 M NaCl were allowed to gel in a 60 mm plastic Petri dish. All the sera were absorbed with sheep erythrocytes and serially diluted. A droplet of each serial dilution of antigen was placed on the gel and allowed to be absorbed. Then 2 ml of a 1:100 dilution of developing antisera were added. After overnight incubation at 4° C the developing reagent was decanted and 1 ml of a 1:10 dilution of guinea pig complement was added. The dishes were then incubated at 37° C for 1 h. The lowest concentration of b4 IgG, still mediating lysis of the Ab-E, was 1 μ g ml⁻¹.

TABLE 3 Rabbit lymphocyte surface immunoglobulin allotypes detected by rosette formation with sheep erythrocytes coated with antibody specific for the b4 and b5 kappa chain allotypes

Rabbit # and genotype	Indicator cells	Inhibitor*	Rosette forming cells (%)
M302 b ⁴ b ⁴	anti-b4-E	—	22
	anti-b5-E	—	0
M341 b ⁴ b ⁵	anti-b4-E	—	2
	anti-b5-E	—	38
	anti-b5-E	b4 serum	33
	anti-b5-E	b5 serum	4
	anti-b5-E	b4, b5 serum	1
	anti-b5-E	anti-b5 antiserum	6
	uncoated-E	—	1

Lymphocytes isolated from heparinised blood by the Ficoll method⁸ were washed thrice with saline, and resuspended to 3×10^6 cell ml⁻¹. Then 0.1 ml of the cell suspension was incubated with 0.1 ml of 1% Ab-E saline containing 0.2% gelatin and 0.05 M NaN₃ at 4° C overnight. Rosetted and nonrosetted lymphocytes were counted in a haemocytometer.

* Inhibitor (5 µl) was added to the cell suspension (0.1 ml) before the addition of Ab-E.

on the antigenic properties of the secreted Ig, the assay can be applied to the enumeration of sub-populations of Ig-secreting cells by exploiting the isotypic, allotypic or idiotype antigenic determinants; for example, allotype secreting cells were enumerated by using erythrocytes coated with the appropriate anti-allotype Ab-E⁷. These immunoglobulin antigen-secreting cells were found in unimmunised individuals, whereas conventional PFC are found only in immunised individuals. Thus, in our PFC assay, an immunoglobulin-secreting cell formed a haemolytic plaque independently from the antibody specificity of the secreted immunoglobulin. This antigen-mediated PFC assay has some distinctive features compared to the conventional antibody mediated PFC assay: (1) the target on the erythrocytes is an antibody rather than an antigen; (2) the lysis of the erythrocytes is mediated by antibody rather than antibody; (3) the cell producing the haemolytic plaque need not be an antibody-secreting cell. Cells other than lymphoid can be tested for secretion, provided that the secreted substance is antigenic. Therefore, this new PFC assay is theoretically a general method for detecting antigen-secreting cells, regardless of the cell type. The method differs from the cytochemical and cytoimmunochemical methods in probing for secretion rather than intracellular presence of antigen.

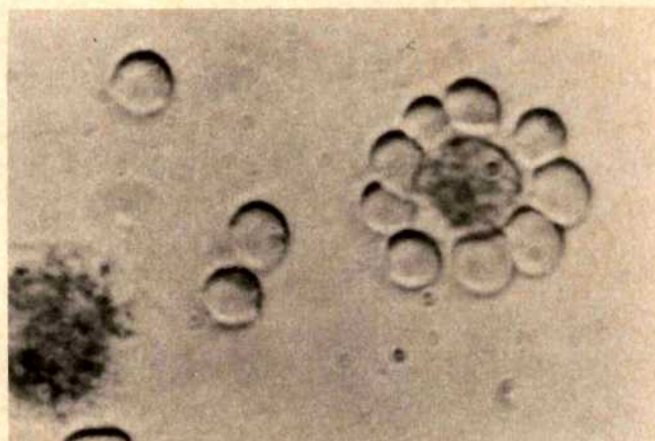


FIG. 3 Microscopic photograph of a rosette formed by a b4 lymphocyte with anti-b4 Ab-E. See Table 3 for experimental details.

Peripheral lymphocytes from homozygous b4 or b5 rabbits were incubated with anti-b4 or anti-b5 Ab-E (Table 3). Lymphocytes from a b4 rabbit rosetted with anti-b4 Ab-E (Fig. 3), but not anti-b5 Ab-E. *Vice versa*, b5 lymphocytes rosetted with anti-b5 Ab-E but not anti-b4 Ab-E or uncoated E. Moreover, this rosette formation of b5 lymphocytes with anti-b5 Ab-E was inhibited by b5 (but not by b4) serum by blocking of the anti-b5 combining sites on the erythrocytes. A similar inhibition was induced by anti-b5 antiserum by masking of the b5 specificities on the lymphocytes (Table 3). The rosetting of antigen bearing cells with Ab-E is a direct simple method for the detection of cell surface antigens and for the enumeration of antigen bearing cells.

In conclusion, we have artificially coupled to erythrocytes various purified antibodies. The specificity of the antibody bound by erythrocytes was retained, as shown by the reactivity of these Ab-E with the corresponding antigen either in soluble form (antigen-induced agglutination and antigen-mediated lysis of Ab-E) or bound to the cell surface (rosette formation with Ab-E). Therefore, Ab-E is a probe for antigen. Accordingly, we have shown that Ab-E can be used in new methods for (1) semi-quantitation of antigen by agglutination, (2) quantitation of antigen by single radial immunohaemolysis (manuscript in preparation), (3) enumeration of antigen secreting cells by a PFC assay⁷, (4) enumeration of cells bearing surface antigen by an immunocytoadhesion method⁷. The same preparation of Ab-E can be used for all of these assays.

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Inheritance of a recombinant HL-A haplotype and genetics of the HL-1 region in man

THE major histocompatibility system of man, designated HL-1, includes a series of closely linked genes of diverse functions¹. HL-1 includes loci controlling two closely linked genes for the segregant series of HL-A antigens, genes responsible for the stimulation in mixed lymphocyte reactions (MLR-S) and possibly a genetic determinant(s) important for the induction of delayed-type hypersensitivity responses (HDR)^{2,3}. Recombination between the genes for HL-A and between HL-A and MLR-S has been reported^{2,3}.

HL-A types and mixed lymphocyte culture reactions of a Caucasian family (designated Hea) reported here provide further information on the relationship between genes in the HL-1 region. The transmission of a recombinant HL-A type

TABLE 1 Mixed lymphocyte cultures of the Hea family

Responder		Stimulators									Negative controls	
Identification	HL-1 genotype or HL-A phenotype	I _{1m}	I _{2m}	II _{4m}	II _{1m}	II _{3m}	II _{5m}	II _{7m}	X _{1m}	X _{2m}	R	S†
Father (DH) I ₁	11-7v/3-7x	709*	6,023	3,303	3,307	3,289	2,115	2,123	5,889	7,557	651	228
Mother (EH) I ₂	3-7y/2-12z	10,280	888	8,648	7,225	9,505	5,101	6,337	13,321	9,408	531	311
Sibling (VH) II ₄	3-7x/2-12z	2,396	4,980	803	2,783	7,985	5,684	5,362	10,556	12,806	598	145
Sibling (KH) II ₁	11-7v/2-12z	1,903	4,921	3,542	469	6,654	3,947	3,629	6,602	10,982	556	272
Sibling (VH) II ₃	11-7v/2-7y	2,893	7,870	16,416	10,446	812	870	822	2,012	16,088	465	342
Sibling (CH) II ₅	11-7v/3-7y	2,955	7,661	12,144	3,267	800	588	826	1,490	15,466	569	139
Sibling (DH) II ₇	11-7v/3-7y	4,410	9,218	16,076	7,973	1,677	1,187	1,545	3,052	3,212	1,782	213
Unrelated (DK) X ₁	2, 10, 8, W18	4,435	10,867	18,227	12,800	1,911	1,855	2,074	960	22,624	1,782	213
Unrelated (MD) X ₂	1, 2, W17, W18	13,574	8,403	21,189	16,205	18,638	14,262	14,155	20,929	523	389	358

* Mean of triplicate determinations of ³H-thymidine uptake by a mixture of 1 × 10⁶ responding and 1 × 10⁶ mitomycin-treated stimulating cells.

† In the negative controls R is the mean of triplicate cultures in c.p.m. of radioactive thymidine uptake of 1 × 10⁶ lymphocytes cultured alone for 120 h. S is the mean of triplicate cultures in c.p.m. of radioactive thymidine uptake of 1 × 10⁶ lymphocytes treated by mytomycin and cultured alone for 120 h.

Enclosed in the rectangle are the values obtained from the reactions of three siblings from the Hea family and one of the unrelated individuals. The low values obtained in those interactions contrast with the stimulation given by other family members.

from the propositus to her progeny confirms the existence of crossing over between the two *HL-A* loci. The MLR reactions between the recombinant and her siblings furnish additional examples of the importance of differences at the second *HL-A* locus for stimulation in mixed lymphocyte culture. Also, the MLR reactions of family Hea provide three examples of strong stimulation between phenotypically identical pairs of subjects, with each pair having one chromosome identical by descent, and presumptive evidence for genetic control of the level of stimulation with cells from an unrelated subject.

HL-A antigens were detected with a standard two-stage, dye exclusion microcytotoxicity test⁴ using 115 well characterised antisera able to detect all defined *HL-A* and most *W* specificities. Tests were repeated at least three times and *HL-A* haplotypes were assigned by conventional methods². Lymphocytes were collected from the subjects into heparinised (25 U ml⁻¹) Pyrex tubes. Part of the sample was transported to the laboratory as whole blood, and part was diluted with an equal quantity of Eagle's minimal essential medium (MEM) and later divided into two fractions which were processed separately. The MLR were carried out as previously described⁵ in triplicate on each of the three cell samples. The final culture volume of 0.2 ml contained 1 × 10⁵ responding and 1 × 10⁵ (mitomycin-treated) stimulating lymphocytes. After 120 h of incubation, cultures were labelled for 18 h with 0.5 μCi ³H-thymidine (New England Nuclear Corp., NET-027 ³H-thymidine-methyl, (NEN) 1.00 mCi ml⁻¹, specific activity 6.10 mmol⁻¹, 0.036 mg). Cells from all donors survived handling when transported as whole blood. When shipped diluted in MEM, cells from one donor were unresponsive, but the others were reactive. Agreement between results of the various fractions, with this one exception, was excellent. The complete data obtained with cells from whole blood are presented in Table 1. The family studied consisted of sixteen members in three generations. Red cell typing gave no evidence of mixed paternity.

The *HL-A* genotypes of the three generations (Fig. 1) show several interesting features. (1) Subject II 3 gave evidence of recombination within the maternally derived haplotypes 3-7/2-12; the recombinant *HL-A* 2-7 haplotype was transmitted to subject III 3. (2) Lymphocytes from the recombinant (II 3) failed to stimulate unidirectional MLR with cells from siblings II 5 and II 7 who differed from her with respect to first segregant series marker *HL-A* 3 (Table 1). Conversely there was stimulation with cells from subject II 1, who differed with respect to the second series marker *HL-A* 12. (Siblings II 5, II 7 and II 1 all share the paternal 11-7 haplotype with II 3 but differ from her in having intact maternal 3-7 or 2-12 haplotypes from which the recombinant was derived.) (3) Lymphocytes from an unrelated subject (DK) stimulated feebly in two tests and failed to

stimulate in the third test with cells from the three MLR identical sibs II 3, II 5 and II 7, while stimulating adequately with all other family members tested. (4) Stimulation developed between subject I 1 (the father) and his phenotypically identical children II 5 and II 7. Stimulation was also observed between the mother I 2 and her phenotypically identical child II 4. To distinguish the *MLR-S* alleles in the *HL-1* haplotypes of the first generation, designations *v*, *x*, *y* and *z* are given in Fig. 1. Alleles introduced in the second generation are not so distinguished. The transmission of the first generation haplotypes can be readily traced.

To explain the strong stimulation observed between a pair of siblings who were genotypically identical for *HL-A*, Plate *et al.* suggested the existence of a locus outside the *HL-A* region and separable from it by recombination⁶. Yunis *et al.* identified another family in which an individual stimulated with his *HL-A* identical sibling^{2,7}. Analysis of the reactions of several families, each including a known recombinant between two segregant series of *HL-A*, showed that stimulation was consistently associated with differences at the second series and not with differences at the first series^{2,8,9}. The recombinant in the family we have reported stimulates with

HL-1 Haplotypes of the Hea Family

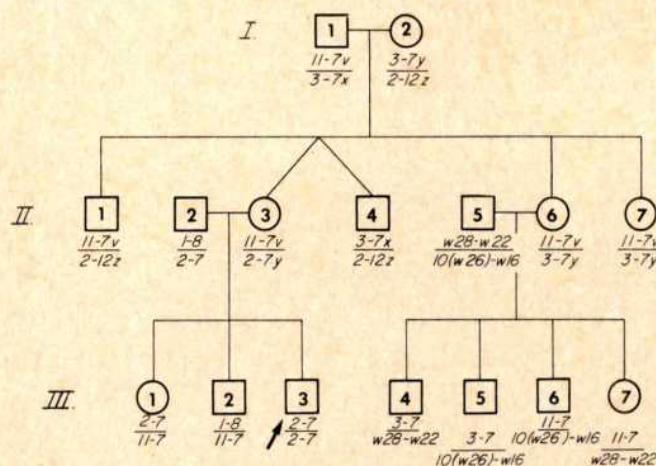


FIG. 1 *HL-1* haplotypes of the Hea family. *HL-1* haplotypes refer to the chromosomal region including two *HL-A* loci, and one *MLR-S* locus (*v*, *x*, *y* and *z*). The hypothetical *HDR* locus is not shown in the pedigree. The arrow identifies the individual to whom the *HL-A* recombinant was transmitted. Numbers under each member of the family represent *HL-A* antigens; the *W* specificities are in conformity with the terminology adopted at the fifth histocompatibility workshop¹¹.

cells from a sibling differing at the second series locus but fails to stimulate with either of the siblings who differ at the first series region. These findings are consistent with the hypothesis that the gene responsible for lymphocyte stimulation, *MLR-S*, is close to the second locus. If this is correct, one would expect the *HL-A* markers to be a poor guide to stimulation in MLR, since the serological tests for *HL-A* do not identify the hypothetical *MLR-S* product. During evolution of the histocompatibility system, recombination between *HL-A* and *MLR-S* should result in a state of equilibrium between the two systems. Therefore, phenotypically identical unrelated individuals would be expected to stimulate frequently, as indeed they usually do². Conversely, one would expect occasional non-stimulation between unrelated subjects regardless of their *HL-A* phenotypes. This family provides three examples of stimulation between phenotypically identical pairs and three examples of non-stimulation or minimal stimulation between phenotypically distinct pairs. One of the individuals (DK) giving minimal stimulation is unrelated, but the pattern of response to her cells correlates with the segregation of the *MLR-S* region of the *HL-1* in the family.

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Initiation of protein synthesis during lymphocyte stimulation

THE stimulation of protein synthesis that accompanies the activation of peripheral blood lymphocytes by phytohaemagglutinin (PHA) has been shown to be due largely to an increase in the rate of initiation of protein synthesis¹. Such an increase could result from either an increase in the availability of initiation factors or of mRNA. This problem can be approached most directly by comparing the ability of cell-free protein synthesising systems from stimulated and

unstimulated lymphocytes to form initiation complexes with ³⁵S-methionyl-tRNA^{Met}. Such studies in reticulocytes have shown that the limitation of initiation occurring during haemin deprivation is accompanied by a marked reduction in the number of methionyl-tRNA-40S ribosomal subunit complexes present, and enabled the elucidation of the mechanisms involved^{2,3}. We show here that the binding of ³⁵S-methionyl-tRNA^{Met} to both 40S subunits and 80S ribosomes is increased after activation of lymphocytes by PHA. These differences can be overcome by the addition of initiation factors from reticulocyte ribosomes, but not by the addition of exogenous globin mRNA.

Lymphocytes were purified from defibrinated pig blood by sedimentation with dextran, filtration through cotton wool to remove phagocytes and centrifugation on ficoll-hypaque step gradients to remove residual erythrocytes. The lymphocytes were cultured at 2×10^6 ml⁻¹ in Eagle's minimal essential medium supplemented with 10% autologous serum. Incubation was for 20-24 h at 37° C, either with or without 3 µg ml⁻¹ purified PHA.

To prepare cell-free extracts, the cells were collected by centrifugation and resuspended at 10^9 ml⁻¹ in 10 mM KCl; 20 mM Tris-Cl pH 7.5; 1.5 mM Mg acetate for 10 min at 4° C. They were disrupted with 10 strokes of a Potter homogeniser. The ionic concentration was adjusted to 80 mM KCl; 25 mM Tris-HCl pH 7.5; 4 mM Mg acetate; 6 mM mercaptoethanol (TKM medium), and the homogenate was centrifuged at 6000g for 6 min. The supernatant was passed through Sephadex G25 equilibrated with TKM medium.

³⁵S-methionyl-tRNA^{Met} was prepared by incubation of discharged rabbit reticulocyte tRNA with ³⁵S-methionine

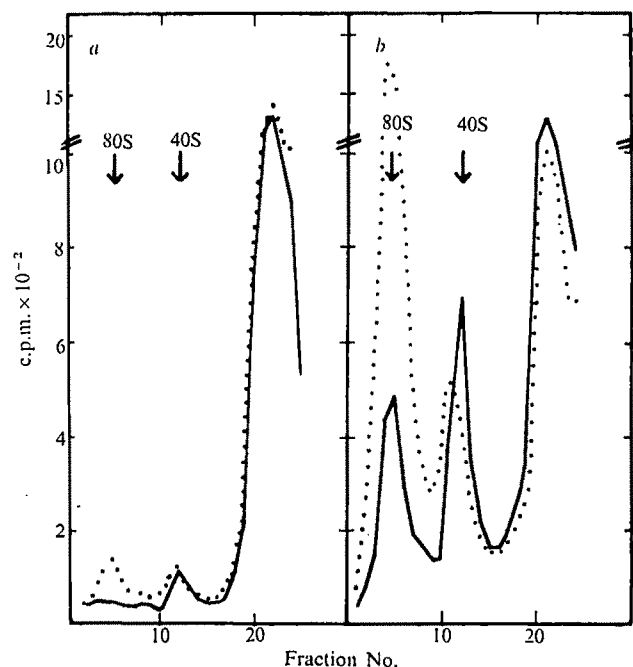


Fig. 1 Effect of globin mRNA on initiation complex formation by extracts from lymphocytes incubated with (b) or without (a) PHA for 24 h. Cell free extracts containing 37 µg RNA were incubated at 30° C for 5 min in 0.2 ml TKM medium supplemented with 1 mM ATP, 0.2 mM GTP, 4 mM creatine phosphate, 0.5 mg ml⁻¹ creatine phosphokinase, a mixture of 19 amino acids excluding methionine, each at 20 µM, and ³⁵S-methionyl-tRNA^{Met} (36,000 c.p.m.). Each incubation was then layered onto a 10-30% sucrose gradient in TKM and centrifuged in the SW 50-1 rotor at 49,000 r.p.m. for 1.75 h. Twenty-six to twenty-eight fractions were collected from each gradient and 1 ml of 2% cetyltrimethylammonium bromide and 1 ml of 0.5 M sodium acetate pH 5.2 containing 0.5 mg ml⁻¹ yeast RNA were added to each. The precipitates were collected on glass fibre filter disks and the radioactivity in each determined. —, Without globin mRNA; ···, with globin mRNA.

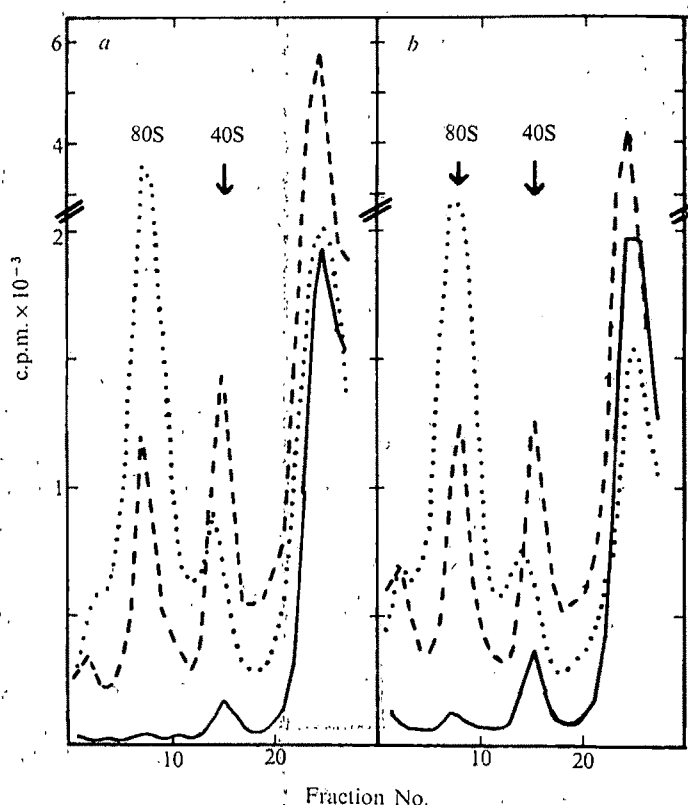


Fig. 2 Effect of reticulocyte initiation factors on initiation complex formation by extracts from lymphocytes incubated with (b) or without (a) PHA for 24 h. The composition of incubation mixtures was as for Fig. 1, except that each contained 19 μ g extract RNA, 72,000 c.p.m. 35 S-methionyl-tRNA Met and, where indicated, 25 μ g reticulocyte initiation factors. Incubation was at 37° C for 2 min. The sucrose gradients included a cushion of 2 M sucrose, and centrifugation was at 42,000 r.p.m. for 2 h. —, Without initiation factors; ---, with initiation factors; ···, with initiation factors and globin mRNA.

and activating enzymes from *E. coli*⁴. Methionyl-tRNA Met is not charged under the conditions used. Globin mRNA was prepared as previously described^{6,6}. Initiation factors were extracted from reticulocyte polyribosomes as described by Schreier and Staehelin⁷. The fraction used was that which eluted from DEAE-cellulose between 0.1 M and 0.25 M KCl, and was concentrated by precipitation with ammonium sulphate.

Extracts prepared from PHA-stimulated lymphocytes incorporated considerably more 14 C-leucine into protein than those from unstimulated cells. In both cases at least 90% of the incorporation was due to the completion of polypeptide chains initiated in the intact cell, as judged by its insensitivity to inhibitors of initiation such as aurin tricarboxylic acid and pactamycin. These results were very similar to those obtained previously with ribosomes from stimulated and unstimulated human lymphocytes⁸.

35 S-methionyl-tRNA Met added to such extracts rapidly became bound to 40S ribosomal subunits, either at 0° C or at 37° C, but there was always more binding to the subunits in the extracts from stimulated cells (Fig. 1). This difference was not due to differential hydrolysis of the 35 S-methionyl-tRNA, as only about 5% was hydrolysed during the incubation period, and the rate of hydrolysis with either extract was not much faster than that with buffer alone.

Little 35 S-methionyl-tRNA became bound to 80S ribosomes in extracts from unstimulated lymphocytes, while there was significant but rather variable radioactivity sedimenting in both the 80S and the polyribosome regions of extracts from PHA-stimulated cells. In some extracts more

methionyl-tRNA was bound to the 80S and polyribosome regions than in the 40S region. The sedimentation of radioactivity in the 80S region was abolished by 10^{-4} M aurin tricarboxylic acid, but not by 3×10^{-3} M cycloheximide.

Addition of globin mRNA increased the binding of 35 S-methionyl-tRNA to the 80S region, but had little effect on the difference between stimulated and unstimulated cell extracts (Fig. 1). The extracts from the PHA-stimulated cells were thus able to utilise the added mRNA more effectively. The amount of radioactivity sedimenting in the 40S region was not much altered by the addition of mRNA, although the peak of radioactivity did sediment a little further down the gradient.

In contrast, the binding of 35 S-methionyl-tRNA to both 40S and 80S regions was greatly increased, and the difference between stimulated and unstimulated extracts almost completely overcome, by the addition of initiation factors from reticulocyte ribosomes (Fig. 2). The binding to the 80S region was abolished by 10^{-4} M aurin tricarboxylic acid or 10^{-5} M edeine. Both these inhibitors prevent the formation of normal mRNA-containing 80S initiation complexes^{9,10}, but do not inhibit the non-specific binding of tRNA to 80S ribosomes, such as occurs at high Mg^{2+} concentrations¹¹. The reticulocyte initiation factor preparation did not contain significant amounts of mRNA, as judged by its inability to cause a 'shift' reaction in sparsomycin-treated reticulocyte lysates¹², so that the mRNA required for the binding of the methionyl-tRNA to the 80S ribosome must be derived from the lymphocyte extracts. Addition of globin mRNA to either type of extract in the presence of reticulocyte initiation factors markedly increases the binding to the 80S region (Fig. 1). In this case the amount of methionyl-tRNA bound to the 40S region, and the amount at the top of the gradient are both reduced, and the slight shift in the sedimentation rate of the radioactivity in the 40S region is more pronounced.

In a previous series of experiments⁸ we showed that after stimulation by PHA, lymphocyte ribosomes contained a higher concentration of those initiation factors required for the translation of the synthetic mRNA poly(U) at low Mg^{2+} concentrations. The results presented here support much more directly the idea that the low rate of initiation in unstimulated lymphocytes is not due simply to a lack of available mRNA, but is due to a lack of one or more of the initiation factors that can be extracted from reticulocyte ribosomes.

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Rosette formation of human erythrocytes on cultured cells of tumour origin and activation of complement by cell membrane

WHEN some cultured cells (OAT cells¹, a cultured cell line from lung cancer) were treated with normal fresh human, guinea pig or rabbit serum and mixed with human erythrocytes (HuE), rosette formation of HuE occurred around the treated cells. We thought initially that the phenomenon might involve natural antibodies and complement because antigen-antibody-complement complexes are known to adhere to HuE by the phenomenon of immune adherence². This possibility was excluded by the finding that rosette formation of HuE around serum-treated cultured cells occurred in the absence of Ca^{2+} , since Ca^{2+} is known to be essential for the reaction of the first component of complement (C1). That the alternative pathway of complement activation³ (Properdin system) might underlie the generation of rosette-forming ability was suggested by the demonstration that Mg^{2+} was an essential requirement.

In the following experiments, gelatin-veronal-buffered saline (GVB) containing 0.1% gelatin was used as the diluent. Three modified diluents were prepared to characterise the role of Ca^{2+} and Mg^{2+} : (1) Ca^{2+} - Mg^{2+} -GVB containing 0.15 mM CaCl_2 and 0.5 mM MgCl_2 ; (2) Mg^{2+} -EGTA-GVB containing 10.5 mM MgCl_2 and 10 mM EGTA (ethyleneglycoltetraacetate); (3) EDTA-GVB containing 10 mM EDTA (ethylenediaminetetraacetate).

OAT cells were treated with diluted serum in Ca^{2+} - Mg^{2+} -GVB, Mg^{2+} -EGTA-GVB or EDTA-GVB (Table 1). After treatment with serum at 30° C for 30 min, cells were washed three times with EDTA-GVB and mixed with HuE. After 1 h incubation at 30° C, the cells were examined microscopically, and the percentage of rosette-forming cells were calculated. Cells which bound two or more HuE were regarded as positive.

In the absence of divalent cations (in EDTA-GVB), OAT cells did not form rosettes with HuE. In the presence of Mg^{2+} , OAT cells developed the rosette-forming ability even in the absence of Ca^{2+} (Table 1). This differential dependence on Mg^{2+} was also found where guinea pig and rabbit serum were used instead of human serum.

Cultured cells derived from Burkitt's lymphoma (Daudi⁴ and P3HR-1 (ref. 5)), HeLa cells and Chang Liver cells were treated with human serum (at a final concentration of 1:15) in the presence or absence of Ca^{2+} and/or Mg^{2+} as described above.

Daudi cells and P3HR-1 cells were able to form rosettes with HuE following treatment with Mg^{2+} -EGTA-GVB but HeLa cells and Chang Liver cells were not (Table 2). In a similar experiment, four cell lines derived from biopsies of nasopharyngeal carcinoma (NPC204, NPC316, NPC415 and NPC428)⁶ all acquired the ability to form rosettes after treatment with human serum in Mg^{2+} -EGTA-GVB. Two primary cultures of acute myeloid leukaemia cells were unable to form rosettes.

OAT cells were also treated with C4-deficient guinea pig serum⁷ in the presence or absence of divalent cations. After treatment in the presence of Mg^{2+} , OAT cells were found to form rosettes of HuE and also to fix C3 molecules on the cell membrane. C3 was detected by use of ¹²⁵I-labelled (Fab')₂ antibody to guinea pig C3. OAT cells treated with

TABLE 1 Rosette formation of HuE around OAT cells treated with human serum in the presence or absence of Ca^{2+} and/or Mg^{2+}

Diluent	Dilution of human serum*			
	1:20	1:60	1:200	0
Ca^{2+} - Mg^{2+} -GVB	56†	48	5	0
Mg^{2+} -EGTA-GVB	43	20	0	1
EDTA-GVB	1	2	0	0

To 0.2 ml 5×10^5 ml⁻¹ OAT in GVB was added 0.1 ml of Ca^{2+} - Mg^{2+} -GVB, Mg^{2+} -EGTA-GVB or EDTA-GVB. To each of these, 0.1 ml of a dilution of human serum in corresponding buffer (Ca^{2+} - Mg^{2+} -GVB, Mg^{2+} -EGTA-GVB or EDTA-GVB) was added. After 30 min incubation at 30° C, the treated OAT cells were washed and resuspended in 0.05 ml of EDTA-GVB and mixed with 0.05 ml of 4×10^7 ml⁻¹ HuE in EDTA-GVB. After 1 h at 30° C, rosette formation of HuE around the treated OAT cells was observed microscopically.

* Final concentration in the above reaction mixture.

† Percentage of OAT cells binding two or more HuE.

C4-deficient guinea pig serum in EDTA-GVB did not form rosettes and did not fix C3. These findings indicated that the cell membrane of cells such as OAT, Daudi, P3HR-1 and NPCs had the ability to activate C3 molecules and to cause them to associate with the cell membrane. It has been reported that trypsinisation of HuE results in loss of the immune adherence receptor⁸. Since trypsinised HuE did not form rosettes on serum-treated OAT cells, a likely interpretation is that the interaction of cell membrane immune adherence receptors and membrane bound C3 is involved in the mechanism of rosette formation. Activation of C3 probably occurs through the alternative pathway³, since the reaction occurred with C4-deficient serum and required Mg^{2+} . An antigen-antibody reaction is unlikely to be involved since sheep erythrocytes sensitised with antiserum did not form rosettes with HuE even after the treatment with human serum in Mg^{2+} -EGTA-GVB. If it is postulated that the process of rosette formation is based on complement activation by the alternative pathway³, the cell membrane of rosette-forming cells may have some molecular configuration similar to that of bacterial lipopolysaccharides which also have the ability to activate the alternative pathway of complement.

To examine the relationship between the rosette-forming ability and virus infection, the following experiment was performed with acute myeloid leukaemia cells. The cells were found to lack rosette-forming ability following treatment with serum in Mg^{2+} -EGTA-GVB. Suspensions of cells were added to spleen extracts of DDD mice suffering from Friend leukaemia, to tissue culture fluid of C3H2K cells infected with Moloney sarcoma virus and to a spleen extract of DDD mice infected with Rauscher virus. Cells were incubated in RPMI 1640 medium containing 20% heat-inactivated α - γ human serum at 37° C under 5% CO_2 . After 12, 36, 60, 84 and 108 h incubation, cells were tested for rosette-forming ability.

TABLE 2 Percentage of rosette-forming cells after treatment with 1:15 human serum in presence or absence of Ca^{2+} and/or Mg^{2+}

Cells	1:15 dilution* of human serum in		
	Ca^{2+} - Mg^{2+} -GVB	Mg^{2+} -EGTA-GVB	EDTA-GVB
Daudi	62%†	49%	0%
P3HR-1	36%	36%	3%
HeLa	7%	0%	0%
Chang liver	11%	1%	0%

To 0.2 ml of 5×10^5 ml⁻¹ cells in GVB was added 0.2 ml of the appropriate diluent; then they were mixed with 0.2 ml of 1:5 dilution of human serum in the corresponding diluent (Ca^{2+} - Mg^{2+} -GVB, Mg^{2+} -EGTA-GVB or EDTA-GVB). After 30 min incubation at 30° C, the treated cells were washed and reacted with HuE as in the legend of Table 1.

* Final concentration in the reaction mixture.

† Percentage of cells binding two or more HuE.

TABLE 3 Effect of addition of virus-rich materials on rosette-forming ability of acute myeloid leukaemia cells

Materials added	Rosette forming ability* after an incubation period of (h)				
	12	36	60	84	108
Friend virus (Spleen extract)	3	0	4	2	3
Moloney sarcoma virus (Tissue culture fluid)	1	0	2	3	25
Rauscher virus (Spleen extract)	0	1	0	2	3
None	0	0	0	1	1

From heparinised blood of an acute myeloid leukaemia patient, a leukaemic cell fraction was collected and suspended in RPMI 1640 medium with 20% heat-inactivated α - γ human serum† at a concentration of 1.5×10^6 ml⁻¹. To 9 ml of the cell suspension, 0.3 ml of Friend virus fraction (spleen extract), 0.6 ml of Moloney sarcoma virus fraction (tissue culture fluid) or 0.3 ml of Rauscher virus fraction (spleen extract) were added. To the control cell suspension nothing was added. Each mixture was separated into 6 tubes each of which contained 1.5 ml. They were incubated at 37 °C under 5% CO₂. After incubation, cells of each tube were washed three times with GVB and resuspended in 0.4 ml of Mg²⁺-EGTA-GVB. To the cell suspension, 0.2 ml of 1:3 dilution of human serum in Mg²⁺-EGTA-GVB were added and the mixture was incubated at 30 °C for 30 min. After washing with EDTA-GVB three times, cells were resuspended in 0.2 ml of GVB and mixed with 0.2 ml of 2×10^6 ml⁻¹ HuE in EDTA-GVB. Rosette formation of HuE on the cells were observed after 1 h at 30 °C.

* Percentage of cells binding two or more HuE.

† From normal human serum of Type O, γ -globulin fraction was removed by 45% saturation of ammonium sulphate. The α - γ human serum was dialysed against saline to remove ammonium sulphate and then dialysed against foetal calf serum to reconstitute dialysable serum factors before use.

following treatment with human serum in Mg²⁺-EGTA-GVB. Cells which had been mixed with material rich in Moloney sarcoma virus became able to form rosettes after 108 h incubation (Table 3). This finding suggested that rosette-forming ability could be brought about by viral infection. If cells able to activate complement non-specifically are either present or are generated *in vivo*, phenomena simulating immunological reactions may occur without reactions of specific antigens and antibodies. Such a mechanism may be involved in the pathogenesis of changes occurring in some of the so-called autoimmune diseases.

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Temperature dependence of cation permeability of dog red cells

It has been suggested that changes in the physical state of phospholipids are responsible for the observed dependence upon temperature and membrane composition of the self-diffusion of sodium ions through lipid vesicles¹ and of diverse membrane transport processes²⁻⁴ some of which are mediated by enzymes^{5,6}. This is supported to some extent by the detection of phase-transitions in both natural membranes⁷⁻¹⁰ and in lipid-extracts¹¹⁻¹³ using X-ray diffraction and other techniques. It seems, however, that only membranes of low cholesterol content exhibit any of the usual characteristics of these transitions^{1,9,12,13}.

We report here that the transport of sodium ions into dog red cells, under isotonic conditions, increases as the temperature is lowered from 38° C and reaches a maximum at 22–25° C. By contrast, the influx of potassium ions is described by a normal Arrhenius relationship between 38° C and about 12° C at which temperature a well-defined minimum is reached. This implies that the fluidity of the lipids in the membranes of these cells, which are rich in cholesterol, does influence the fluxes of sodium and potassium. Furthermore, the disparity between the effects of temperature on the fluxes, which are mainly passive in this system, indicates that two independent processes are involved in the ion-transport mechanisms.

Blood was drawn from the jugular vein of unanaesthetised Beagles into syringes wetted with heparin. Within a few minutes it was chilled to 4° C and centrifuged at about 1900g (MSE Minor). Plasma and white cells were aspirated and the red cells were washed three times at 4° C with a solution containing NaCl 142.2 mM, KCl 5.0 mM, Na₂HPO₄ 5.2 mM, NaH₂PO₄ 0.8 mM, CaCl₂ 1.0 mM, MgCl₂ 0.25 mM, glucose 5.0 mM and 1% (w/v) bovine serum albumin (Sigma, fraction V). The washed cells were suspended (5 ml packed cells to 25 ml of bathing medium) in Erlenmeyer flasks which were placed on ice. ¹³¹I-human serum albumin (Radiochemical Centre, Amersham) was passed through an ion exchange resin (Deacidite FF-1P, Permutit) before it was made up in isotonic buffer and added to each suspension (2 μ Ci ml⁻¹).

Cell suspensions were shaken in a water bath at 38° C for 30 min and then transferred to shaking water-baths at the temperature of the influx of ²⁴Na and left for a further 30–45 min at 38° C, or for 1–2 h at lower temperatures. At the end of these incubation periods, 0.2–1.0 ml of buffer solutions containing either ²⁴Na or ⁴²K (Radiochemical Centre, Amersham) were added to each suspension (10 μ Ci ml⁻¹) at 15 s intervals after which the suspensions were agitated vigorously for 2 min. At various times thereafter, 4 ml samples of each suspension were pipetted into centrifuge tubes pre-cooled in ice-water. These were transferred to the cold room (4° C) and centrifuged for 3–5 min. Samples of the supernatants were saved and the remainder aspirated. 100 μ l volumes of the packed cells were taken using a gas-tight Hamilton syringe with a Chaney adaptor (Reno, Nevada) and lysed by addition to 2 ml of water in Pyrex test-tubes. The Hamilton syringe was calibrated and served as the primary reference for the volume of packed cells in each lysate; it was rinsed several times with detergent solution (2% Triton X-100) and water between each pipetting. At temperatures between 20° C and 38° C, samples of red cell suspensions were taken at 30–60 min intervals throughout 3–5 h, but below 20° C samples were taken at less frequent intervals spaced over 12–15 h. The radioactive supernatants were diluted and prepared for γ -counting in the same way as the lysates. The activities of ²⁴Na (or ⁴²K) and ¹³¹I were measured in a two-channel γ -counter (Packard, Model 3002 or LKB Wallac 80000) and the appropriate corrections for

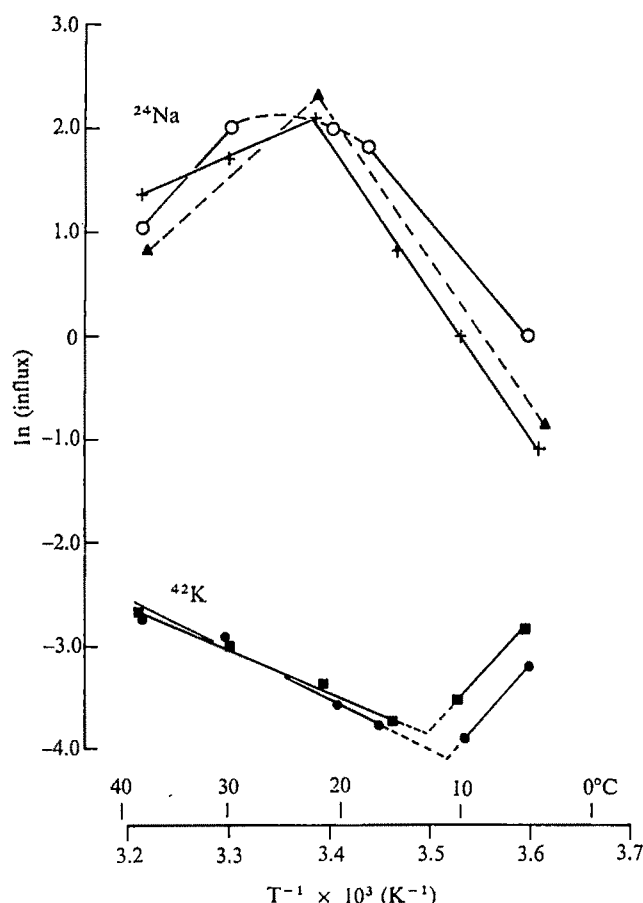


Fig. 1 Arrhenius plots of steady-state fluxes of sodium and potassium in dog red cells. The values of the fluxes (meq per l cells h^{-1}) at $38^{\circ}C$ were 2.28 ± 0.35 (+), 3.96 ± 0.10 (Δ) and 2.87 ± 0.25 (O) for sodium, 0.0645 ± 0.0012 (\bullet) and 0.0703 ± 0.0049 (\blacksquare) for potassium. Blood from a different animal was used in each set of flux measurements. The errors arise mainly from the standard errors of the regression lines fitted by the method of least squares to 3–5 points on an uptake curve. In one experiment (O—O) the cells were not pre-incubated at $38^{\circ}C$ as described in the text, but were left in ice for a few hours after washing, and then gently agitated for 1–2.5 h at the temperature of ^{24}Na -uptake before the addition of tracer. The least squares fit to the ascending limb of (+—+) gave a negative apparent activation energy for sodium influx of 8.7 ± 0.3 kcalorie mol^{-1} and for the descending limb a positive value of 28.2 ± 0.6 kcalorie mol^{-1} , one point at $22.4^{\circ}C$ appeared to be common to both limbs of the Arrhenius plot. The activation energies for potassium influx between $38^{\circ}C$ and $15^{\circ}C$ were 8.5 ± 0.3 kcalorie mol^{-1} (\blacksquare — \blacksquare) and 9.2 ± 1.2 kcalorie mol^{-1} (\bullet — \bullet); below the 'transition' temperature at 11 – $13^{\circ}C$ the value was about -22 kcalorie mol^{-1} .

^{24}Na (or ^{42}K) activity in the ^{131}I channel were made. Finally the concentrations of haemoglobin, sodium and potassium in each lysate were measured. After correcting for tracer in the trapped extracellular volume of the packed cells, it was possible to calculate the ^{24}Na or ^{42}K uptake in terms of activity per unit cell volume, the relative volume of the cells from the intracellular concentration of haemoglobin, and the intracellular concentrations of the cations from which net movements of Na and K could be estimated to check if the cells were in the steady-state. This method for the measurement of tracer uptake in cell suspensions has obvious advantages over the more conventional techniques which involve repeated washing of the cells in cold isosmolar $MgCl_2$ (ref. 14) or $NaSCN$ (ref. 15) solutions to remove 'extracellular' tracer.

Provided that extracellular tracer had exchanged with less than 20% of the intracellular cation, the uptake of ^{24}Na or ^{42}K was usually linear with time except for a small deviation

at times less than about 20 min. This initial curvature probably represents the penetration of tracer into a small space (less than about 1.5%) beyond that accessible to ^{131}I -albumin yet still extracellular. Fluxes (meq per l cells h^{-1}) of sodium and potassium were calculated from the ratio of the slope of the regression line fitted to the uptake of the tracer and the extracellular specific activity of the cation.

The temperature dependence of the cation fluxes is illustrated by the Arrhenius plots in Fig. 1. It was surprising to find that the sodium flux was maximal at about $22^{\circ}C$ at which temperature the flux was three times greater than at $38^{\circ}C$. By contrast, the potassium flux decreased as the temperature was lowered from $38^{\circ}C$ to about $12^{\circ}C$, and then passed through a minimum. At all temperatures the cells remained at constant volume throughout the uptake of the tracers as indicated by the measurements of intracellular haemoglobin. Within experimental error the intracellular cation contents did not vary significantly with temperature, although cell volume at 18 – $22^{\circ}C$ was slightly ($\leq 3.7\%$) greater than at $38^{\circ}C$. This difference in cell volume, however, could not account for the results as it is established that as dog red cells swell sodium flux decreases and potassium flux increases^{16–18}.

Vieira *et al.*¹⁹ have shown that the apparent activation energies for both the hydraulic conductivity and the diffusion permeability of water in dog red cells are constant from $37^{\circ}C$ to $7^{\circ}C$. Taken in conjunction with the present results, this implies that the translocation of water, potassium and sodium across the membrane of this type of cell takes place by means of independent mechanisms and that the transport of either cation cannot occur through water-filled pores in the membrane. Furthermore, the lack of coupling between the effects of temperature on the transport of the different species makes it unlikely that any long-range reorganisation of the membrane lipids occurs when the fluxes of sodium and potassium are maximal and minimal respectively. It seems more likely that changes in the conformation of localised lipid-protein complexes are involved.

Davson and Reiner²⁰ first showed that the net efflux of sodium from cat red cells suspended in KCl solutions was maximal at around physiological temperatures and, more recently, this has been confirmed in cells under steady-state conditions²¹. Therefore, while cat and dog cells have a number of characteristics in common in that they are sodium-rich, have a low Na-K ATPase activity²² and have a cation transport system that is dependent on cell volume^{16–21,23}, it would seem that there is a marked shift in the temperature at which the permeability of the membranes to sodium is maximum. It may be possible to relate this to the small but definite differences in the lipid composition of the membranes²⁴.

It is worth noting perhaps that a 'suggestion' of a phase-transition at about $22^{\circ}C$ in human red cell ghosts has been found recently using a dilatometric technique²⁵.

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Deficiency of two enzymes of galactose metabolism in kangaroos

THE starting point of the investigation reported here was the observation by one of us of diarrhoea and occasional cataract formation in bottle-fed orphan pouch-young kangaroos. As kangaroo milk has a very low lactose content¹⁻³ and the intestinal lactase content of pouch-young kangaroo is also low⁴, we thought that these marsupials may not utilise galactose, present mainly as polygalactan in milk, by the usual pathway involving galactokinase (E.C. 2.7.1.6) and galactose-1-phosphate uridyl transferase (E.C. 2.7.7.12) (transferase). With this postulate, and the role of deficiency of these two enzymes in human cataractogenesis in mind, galactokinase and transferase were assayed in haemolysates from the grey (*Macropus giganteus*) and the red kangaroo (*Megaleia rufa*).

Blood was collected from lateral tail veins of unanaesthetised adult male and female kangaroos, and by venepuncture from normal adult humans of both sexes. Haemolysates were pre-

TABLE 1 Erythrocytic galactokinase and galactose-1-phosphate uridyl transferase of grey and red kangaroos

Species	Galactokinase ($\mu\text{mol galactose}$ phosphorylated $\text{h}^{-1} \text{ml}^{-1}$ RBC)		Galactose-1-phosphate uridyl transferase (U g^{-1} haemoglobin)	
	No.	Mean \pm s.d.	No.	Mean \pm s.d.
Grey kangaroo	22	0.039 ± 0.013	19	2.2 ± 1.7
Red kangaroo	21	0.033 ± 0.009	20	10.6 ± 3.5
Human	200	0.213 ± 0.028 (7)	61	21.4 ± 3.2

Statistically, galactokinase and transferase values from both species of kangaroos were significantly different ($P < 0.001$ in all comparisons) from human values. Galactokinase was not significantly different between two species of kangaroos, but transferase was so ($P < 0.001$). RBC, red blood cells.

TABLE 2 Amount of methaemoglobin reduced in 3 h with either galactose or glucose as substrate by erythrocytes from grey and red kangaroos

Species	No.	% Methaemoglobin reduced	
		Galactose substrate	Glucose substrate
Red kangaroo	6	5.0 ± 1.8	15.2 ± 5.3
Grey kangaroo	6	1.9 ± 1.4	11.4 ± 3.6
* Human	14	10.2 ± 2.0	28.0 ± 5.2

* Human values as reported earlier⁵.

pared and galactokinase was assayed as previously described⁵. Transferase was determined using Sigma Kit (Sigma), by the UDPG consumption test⁶, as described in *Sigma Technical Bulletin* No. 600-UV. A normal blood sample was always used as control for each batch of assays for both enzymes. To study the overall utilisation of galactose by erythrocytes, a modified methaemoglobin reduction test was performed for 3 h as described earlier⁵, with galactose as substrate; glucose was used as substrate for comparison.

The results of enzyme assay are shown in Table 1. In both red and grey kangaroos, galactokinase activity was about one-sixth of the normal human value. It should be noted that red cell galactokinase activity in both species was much lower than in various other mammals⁵. A distinct difference between the two species was observed with regard to transferase, in that the grey kangaroo almost completely lacked this enzyme, whereas the red kangaroos had levels approximating those in humans heterozygous for galactose-1-phosphate uridyl transferase deficiency. Erythrocytic haemolysates from seven of the nineteen grey kangaroos had enzyme activity of less than 1U, and four of these had no activity at all. In both species, similar values of galactokinase and galactose-1-phosphate uridyl transferase were found in males and females.

The combined effect of the deficiency of both galactokinase and transferase in grey kangaroos and of galactokinase, but not transferase, in red kangaroos is suitably demonstrated by measuring rates of methaemoglobin reduction with galactose as substrate (Table 2). While both grey and red kangaroos were substantially less able to reduce methaemoglobin than humans, this deficiency was more pronounced in grey than in red kangaroos. The faster rates of methaemoglobin reduction with glucose as substrate indicate that the galactose pathway limits the rate of methaemoglobin reduction in both kangaroos and humans.

We are presenting these results to draw attention to the possibility that other marsupials may prove to be deficient in enzymes of galactose metabolism, and that such animals may provide useful models for galactose cataractogenesis. Mathai *et al.*⁷ investigated the possibility of using California sea lions (*Zalophus californianus*), whose milk is completely deficient in lactose, for the study of the pathogenesis of galactosaemia in man, but found that their erythrocytes were not deficient in either galactokinase or transferase activity, possibly due to the high galactose content of mucopolysaccharides in various aquatic organisms on which they feed. In view of their almost complete lack of transferase, grey kangaroos may be a very useful animal model for the study of galactosaemia in man.

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Mammatropic effect of prolactin enhanced by thyroidectomy

THE role of thyroid hormones in relation to mammary gland structure and function has received sporadic attention. It has been shown in the rat that thyroid hormones are not necessary for mammary development¹ and that in ovariectomised, adrenalectomised, hypophysectomised rats, prolactin and growth hormone are the principal hormones responsible for lobulo-alveolar growth². The slight stimulatory effect of thyroid hormones on mammary growth and lactation in animals has been attributed to their general metabolic effects^{3,4}. There are, however, a few contrary reports in the literature describing greater mammary development in rats rendered hypothyroid^{5,6}, which have remained unexplained. The present study was undertaken to clarify the relationship between thyroid hormones and mammary growth, with special reference to prolactin. Interest in this field was primarily aroused by several clinical and epidemiological reports associating thyroid hypofunction with breast cancer^{7,8}.

Two separate experiments were carried out. In the first, thirty-six Sprague-Dawley female rats, weighing about 200 g, were divided into six equal groups. They were fed on a commercial diet and given tap water *ad libitum*. All rats, including controls, were given oestradiol (8 µg subcutaneously s.c.) daily from day 1 of the experiment to day 10 inclusive¹⁰. In addition, rats in each group were treated as described in the legend to Fig. 1. The animals were killed on day 16. The left inguinal mammary glands were removed, fixed in buffered formal saline, processed for histology, and the sections stained with haematoxylin and eosin (Fig. 1). In oestrogen-primed rats, thyroidectomy induced a marked stimulation of mammary development, which was completely suppressed by replacement doses of thyroxine. A marked suppression of mammary stimulation also occurred when 2-bromo-α-ergocryptine (CB154), a compound known to reduce but not abolish prolactin secretion⁹, was administered. By raising the prolactin level with perphenazine¹⁰, the mammary glands of thyroidectomised rats could be still further stimulated with the production of macroscopic secretion.

In the second experiment twenty Sprague-Dawley rats, weighing about 200 g, were divided into two groups of ten and fed and watered as in the first experiment. All rats were ovariectomised and thyroidectomised on day 1 of the experiment. Each rat received daily injections of oestradiol (1 µg s.c.), progesterone (3 mg s.c.) and thyroxine (2 µg per 100 g body weight i.p.) from day 1 to day 15. One group received bovine thyrotropic hormone daily (TSH:1.5 IU (s.c.)) from day 11 to day 15, the second group remained as untreated controls. All rats were killed on day 16 and the mammary glands removed and processed as in the first experiment. Histological examination showed no difference between the two groups.

The marked enhancement of mammary development by thyroidectomy in the first experiment might be explained

by the following possibilities: (1) thyroidectomy causes an increase in the secretion of prolactin and/or growth hormone; (2) thyroidectomy causes an increased secretion of ovarian oestrogens which in turn stimulates prolactin secretion; (3) TSH, which is increased after thyroidectomy, has mammatropic properties; (4) prolactin exerts a greater mammatropic effect in the absence of thyroid hormones.

The first two possibilities are unlikely as measurement of prolactin and growth hormone levels in the blood of hypothyroid rats, by sensitive radioimmunoassay methods, has shown that there is no significant change in the prolactin level, whereas there is a dramatic fall in the level of growth hormone¹¹. Previous observations by Leonard and Reece⁶ of greater mammary development in thyroidectomised-ovariectomised rats compared to ovariectomised controls, also excludes the involvement of ovarian hormones in this phenomenon. The third possibility that TSH may have mammatropic properties is proved unlikely by the second experiment. Therefore, it seems that the fourth possibility is the most likely: that in the absence of thyroid hormones, the mammatropic effect of a normal level of endogenous prolactin is markedly increased and that further stimulation occurs when the prolactin level is raised by pharmacological means.

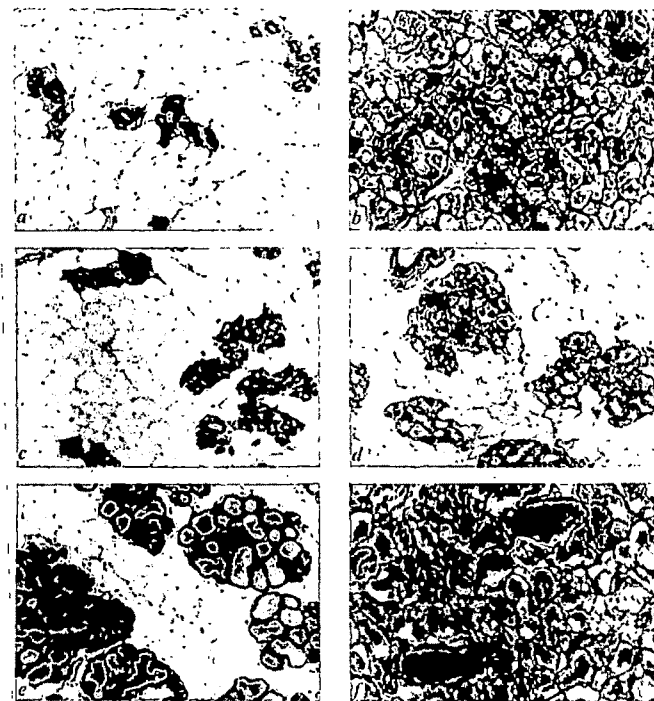


FIG. 1 Photomicrographs of sections of mammary gland showing the relationship between the effects of prolactin and thyroxine on mammary development in the rat. Stain: haematoxylin and eosin $\times 52$. All rats, six per group, were given oestradiol 8 µg s.c. daily from day 1 to day 10 inclusive and killed on day 16. In addition, rats in each group were treated as indicated: a, Control group (no additional treatment) showing clusters of ducts amidst mammary fat with little alveolar formation. b, Thyroidectomy (on day 1), marked duct and alveolar development replacing mammary fat. Alveoli contain secretion. c, Thyroidectomy (on day 1) + thyroxine (in alkaline solution) 2 µg per 100 g body weight per day i.p. from day 1 to day 15 mammary development comparable with the control group. d, Thyroidectomy (on day 1) + 2-bromo-α-ergocryptine (in 70% ethanol) 400 µg per 100 g body weight per day i.p. from day 1 to day 15. Reduction in the quantity of developed glandular tissue compared with b occupying a lesser volume of mammary fat. e, Perphenazine 5 mg per kg body weight per day s.c. from day 11 to day 15. Marked glandular development with alveolar secretion. f, Thyroidectomy (on day 1) + perphenazine 5 mg per kg body weight s.c. from day 11 to day 15. Extreme mammary development, alveoli distended with secretion. Total replacement of mammary fat.

If one assumes that the converse holds true, that excess thyroxine inhibits the mammatropic action of prolactin, then the phenomenon reported by Meites and Kragt¹² might be explained in the light of my findings. These workers observed prolactin induced mammary development around an ectopically transplanted pituitary in immature hypophysectomised rats, which was unexpectedly abolished when thyroxine was administered, despite an increase in weight of the animals. Although thyroid hormones are galactopoietic in physiological doses¹³, suppression of lactation is known to occur in the rat¹⁴ and mink¹⁵ when pharmacological levels of thyroactive substances are administered; large amounts of thyroid hormones being required presumably to block the elevated prolactin level during lactation.

A prolactin-thyroxine antagonism is also known to occur in some lower vertebrates. In urodele development, thyroxine is responsible for 'land drive' and prolactin for 'water drive', the effect of one hormone being reversed by the other under experimental conditions¹⁶. Etkin has recently drawn attention to a similar antagonism in amphibian metamorphosis¹⁷; repeated injections of prolactin in larval tadpoles prevents thyroxine-induced metamorphosis into adult structures until additional thyroxine is given¹⁸. Bern and Nicoll¹⁹ have suggested that this antagonism takes place at a peripheral tissue level. The present experiment indicates a similar antagonism at the level of the rat mammary epithelium.

If hypothyroidism sensitises the rat mammary epithelium to the action of prolactin, this condition should also enhance 7,12-dimethyl-benz(a)anthracene (DMBA)-induced mammary tumorigenesis, which is prolactin dependent²⁰. Shellabarger²¹ obtained twice as many tumours in rats rendered hypothyroid by propylthiouracil (PTU) administered for only two weeks before DMBA, compared with controls; but continuous PTU treatment, maintained after DMBA, suppressed tumour production. Therefore, hypothyroidism enhances tumour initiation but cannot promote tumour growth, presumably due to disappearance of growth hormone from blood²².

The antagonism between prolactin and thyroxine at the level of the rat mammary gland is a new finding the mechanism of which remains to be investigated. In view of a similar observation in some lower vertebrates, a wider implication of this phenomenon cannot be excluded. It is possible that the level of thyroid hormones in blood determines the 'set point' for the mammatropic effect of prolactin. Reports of massive virginal breast hypertrophy in association with hypothyroidism²³ and objective and subjective improvement after thyroid therapy in women suffering from mammary dysplasia^{23,24} point to possible clinical evidence of a similar phenomenon.

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Oestradiol inhibition of collagenase role in uterine involution

THE uterus of the rat provides an ideal organ for the study of collagen breakdown; during the *post partum* involution of this organ the half-life of the uterine collagen is reduced to 24–30 h. Previous studies have shown that this rapid breakdown of collagen in the rat uterus is accompanied by extensive phagocytosis of collagen^{1–3}, elevated lysosomal enzyme levels⁴, the presence of hydroxyproline in lysosome-like particulates, and an increase in the concentration of a collagenolytic cathepsin which digests collagen at acid pH (ref. 5). All of these findings are consistent with the intracellular ingestion and digestion of the extracellular collagen fibres.

None of these findings, however, gives a clear explanation of how the fibres are broken into fragments and brought inside the cell, nor do they allow an evaluation of the relative contributions of intra and extracellular digestion processes. It has been suggested that an extracellular collagenase active at neutral pH is required for the first step in collagen breakdown⁶. Such a collagenase has been demonstrated by culturing fragments of rat uterus; the collagenase is secreted into the culture medium from which it can be purified and characterised⁷. Enzyme is produced only by tissue from involuting uteri⁸. This method enables measurement of the potential of the tissue for collagenase elaboration but it does not reveal how much enzyme is actually present in the tissue. Recently, we have developed a method which enables the direct measurement of collagenase in the particulate fraction of uterine homogenates⁸. We have shown that the method is relatively specific for collagenase⁹ and that the enzyme being measured is a true collagenase¹⁰. Here we report findings obtained by applying this assay to the rat uterus in various stages of pregnancy and involution.

It has further been shown that oestradiol is a potent and selective inhibitor of collagen breakdown in the uterus¹¹. In searching for explanations for this effect it was shown that lysosomal enzyme levels, acid collagenolytic cathepsin activity, and collagen peptidase activity remained unaffected⁵. It has also been shown that oestradiol does not affect collagenase production by uterine slices in culture¹². We investigated whether oestradiol affected uterine collagenase when the hormone was administered to the intact rat.

TABLE 1 Collagenase activity in the involuting uteri of control and estradiol-treated rats

Experimental group	No.	Hydroxyproline (mg per uterus)	Collagen digestion (%)	Collagen digestion (μ g hydroxyproline per uterus)
Non gravid	5	1.36 \pm 0.05	2.0 \pm 1.0	27 \pm 14
Pre partum (1 d)	5	6.89 \pm 0.90	0.70 \pm 0.68*	49 \pm 48
Parturition	6	8.80 \pm 2.20	2.9 \pm 1.1	226 \pm 113
Post partum controls:				
Day 1	5	8.10 \pm 1.39	5.1 \pm 2.3	396 \pm 129
Day 2	8	4.63 \pm 0.39	14.0 \pm 2.8	658 \pm 182
Day 3	6	1.73 \pm 0.37	12.9 \pm 2.9	220 \pm 52
Day 4	7	0.89 \pm 0.35	8.2 \pm 1.8	77 \pm 36
Oestradiol-treated:				
Day 1	5	8.08 \pm 1.32	4.7 \pm 1.4	368 \pm 84
Day 2	8	4.88 \pm 0.49	4.6 \pm 0.9†	237 \pm 120†
Day 3	6	3.94 \pm 0.34†	5.3 \pm 0.8†	207 \pm 30
Day 4	5	3.80 \pm 0.89†	2.1 \pm 1.0†	73 \pm 20

All values given as mean \pm s.d.

* This value differs from parturition by $P < 0.01$ and from non-gravid by $P < 0.05$.

† Value significantly ($P < 0.001$) less than relevant control.

Pregnant female rats were purchased from Sprague-Dawley Farms, Madison, Wisconsin, at the 17th day of gestation and were maintained on Purina rat chow. The time of delivery was recorded and the young were allowed to nurse. Injections of 17β -estradiol (Mann Research Laboratories, New York) were administered intraperitoneally as a suspension of the hormone in 0.9% NaCl (100 μ g in 0.1 ml.) shortly after delivery was complete and on each successive day. Uteri were removed and dissected free of mesentery and placental sites. Preparation of tissue homogenates and determination of collagenase activity in the 6000g pellets were as previously described⁸. The collagen of the uterus served as the substrate for the collagenase, and EDTA blanks were used in every case.

The major findings are presented in Table 1. The % collagen digestion was measured in the 6000g pellet from 0.2 g wet uterine tissue in a total volume of 2.0 ml. All of the pellets at all points contained approximately the same amount of collagen (about 700 μ g hydroxyproline) with the exception of the 3 and 4 d controls (about 500 μ g). Therefore, as substrate and tissue weight were constant, the results are essentially a measure of enzyme concentration. The total digestion per uterus (final column) was calculated by multiplying the % digestion by the total hydroxyproline content for each sample. This provides an estimate of the total units of enzyme per whole organ.

Very little collagenase activity can be found in non-gravid uteri and in uteri 1 d before parturition. Collagenase rises by the time of parturition and then rapidly increases to a peak of concentration at day 2-3 and of total units at day 2 post partum. By 4 d, involution is almost complete; there is enough enzyme to digest 8% of the collagen, but the uterus contains very little remaining collagen. These data on collagenase activity correlate closely with previous studies on the rate of collagen breakdown in the involuting uterus¹¹. The maximum rate of hydroxyproline loss occurs between day 1 and 3 (Table 1). The data also agree with the finding of Jeffrey *et al.*⁶ that maximum production of collagenase in cultures of uterine tissue is observed with tissue removed at 1-2 d post partum. The correlation of activity with rate of collagen loss is not perfect, because at parturition there is an appreciable level of collagenase activity but almost no collagen degradation for the first 24 h. It should also be noted that the digestion of collagen during the 20 h assay is only about one-fifth that which occurs *in vivo* during the same period (such as day 2). The system *in vitro*, however, has been diluted 10-fold and optimum conditions may not obtain. On the other hand, other pathways for collagen breakdown may also be operative *in vivo*.

Oestradiol treatment strongly inhibits collagen breakdown. The values of hydroxyproline per uterus show that the main inhibitory effect occurs between day 2 and 3 (Table 1). It is just at the beginning of this period that the collagenase levels of oestradiol-treated animals vary most markedly from controls. At day 2 the oestradiol-treated tissue has only about one-third the control value of collagenase, whether expressed as concentration or as total units per uterus. The collagenase content never increases over that found at day 1; the concentration continues to remain low through day 4. Whereas the total activity per uterus approaches control values on days 3 and 4, the low enzyme concentration and the high collagen content prevent the treated uteri from maintaining a normal rate of collagen breakdown.

Oestradiol does not act by altering the collagen substrate: solubility and susceptibility to added uterine collagenase, acid collagenolytic cathepsin, and trypsin are unaffected. Uterine homogenates contain inhibitors which are normally discarded with the supernatant before the assay. Figure 1 shows that the supernatants from oestrogen-treated rats are, if anything, even less inhibitory than the control supernatants. Moreover, a study of the supernatants by the method of Sakamoto *et al.*¹² indicates that very little collagenase is present. All evidence

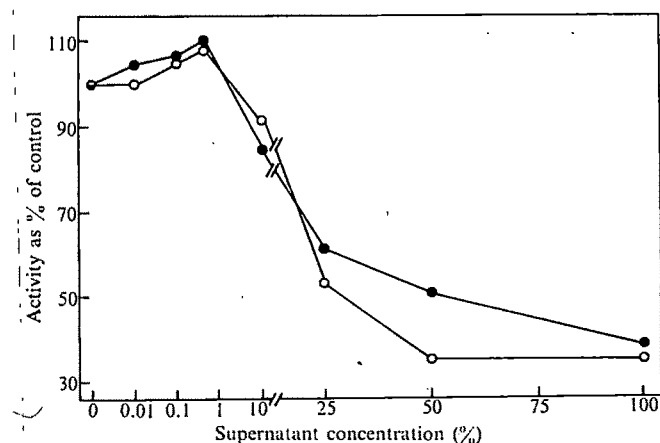


FIG. 1 Effect of 6000g supernatants of uterine homogenates on collagenase activity in uterine pellets. The preparation of supernatants and pellets and the enzyme assays are as previously described⁸. The supernatants were adjusted to pH 7.6 and added to control pellets to produce the final concentration shown. ○, Supernatant from 2 d post partum uterus of control rat; ●, supernatant from corresponding uterus of oestradiol-treated rat. A digestion of 100% of the control value corresponds to 15.2% digestion of the collagen.

to date points to the presence of fewer active molecules of collagenase in the oestrogen-treated uteri.

A recent report by Jeffrey *et al.*¹² showed that progesterone added to cultures of rat uterine tissue inhibits collagenase production, whereas oestradiol has no effect. This suggests that the oestradiol effects observed in the intact rat may not be directly mediated by the uterus.

It has been shown by Yoshinaga *et al.*¹⁴ that the rat ovaries at the time of parturition produce almost 1 µg of oestrogen per day. We showed earlier¹¹ that 1 µg of oestradiol per day clearly inhibited collagen breakdown so that by 4 d the treated uteri contained twice as much collagen as the controls. The present dose of 100 µg was selected to maximise this effect, leaving four times as much collagen at 4 d.

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Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone

THYROTROPIN-RELEASING hormone (TRH) (pyroglutamyl-histidyl-proline-amide) has been reported to ameliorate mental depression^{1,2}. The mechanism of action of the tripeptide in this condition is unknown. It may be connected with changes in the activity of noradrenergic or 5-hydroxytryptaminergic neurons which are thought to be involved in depressive states^{3,4}. Therefore, we have investigated the action of TRH on the turnover of biogenic amines in rat brain.

Male albino rats of 200 g, Wistar origin, were injected intraperitoneally with TRH. Thereafter noradrenaline, dopamine, 5-hydroxytryptamine (5-HT) as well as their corresponding main metabolites 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG, isolated as the sulphate ester),

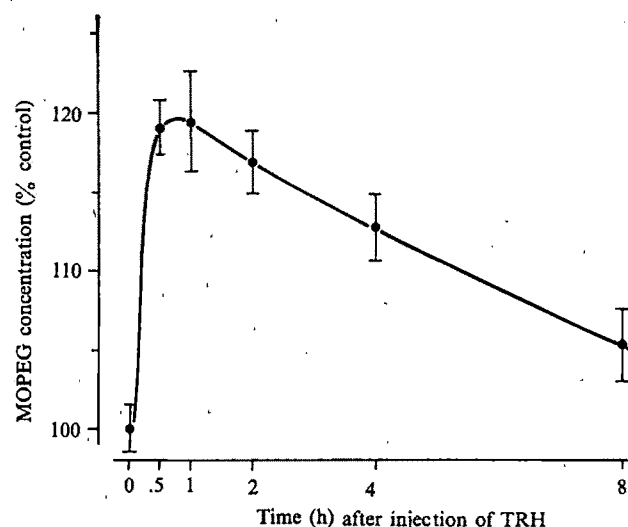


FIG. 1 Effect of intraperitoneal injection of 10 mg kg⁻¹ TRH on the content of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) in the cerebral cortex of rats. Each value is expressed in percent of untreated controls (95.7 ± 2.9 ng g⁻¹ = 100%) and represents the average with s.e.m. of results from twenty to forty determinations per group performed in four to eight experiments. Each determination was carried out in two pooled cerebral cortices. Significance against controls: MOPEG concentration 0.5, 1, 2 and 4 h after TRH injection. $P < 0.001$ (Student's *t* test).

homovanillic acid and 5-hydroxyindoleacetic acid were measured in whole brain and/or various brain parts⁵ by spectrophotofluorimetric methods⁶⁻¹⁰. In other experiments the action of triiodothyronine (T₃) on the levels of MOPEG in different brain regions was determined. In animals thyroidectomised 2 d before TRH injection, estimations of MOPEG were carried out in the same cerebral regions. In addition, the effect of TRH on the accumulation of ¹⁴C-noradrenaline and ¹⁴C-dopamine in the whole brain was measured in rats injected with L-3-¹⁴C-tyrosine (30 µg in 20 µl saline; specific activity 53.9 mCi mmol⁻¹) through a permanent cannula implanted in one lateral cerebral ventricle¹¹. Labelled noradrenaline and dopamine were isolated by adsorption on alumina, separated by column chromatography on Dowex 50 × 8 and estimated in a liquid scintillation counter^{12,13}. Normal and thyroidectomised rats not injected with TRH served as controls. In the thyroidectomised animals the concentration of total thyroxine in the blood serum (measured by competitive protein-binding analysis¹⁴) had dropped to about one-fifth of its normal value and did not rise following TRH injection.

Two hours after administration of 10 mg kg⁻¹ TRH to normal rats, the cerebral content of noradrenaline, dopamine, 5-HT, homovanillic acid and 5-hydroxyindoleacetic acid did not significantly differ ($P > 0.05$) from that in untreated controls (% values after TRH: 101.0 ± 1.5%, 99.3 ± 2.9%, 102.0 ± 2.5%, 104.0 ± 3.2% and 98.7 ± 3.2%, respectively; controls: 100%). The concentration of MOPEG was, however, significantly increased ($P < 0.01$) in the whole brain as well as in the various brain regions (Table 1). In the cerebral cortex the action of TRH lasted for at least 4 h, maximal values of MOPEG (about 120% of controls) being reached between 30 min and 1 h after injection (Fig. 1). The concentration of MOPEG in various brain parts of thyroidectomised rats did not differ from that seen in controls with the exception of mesencephalon plus medulla oblongata where MOPEG concentrations were significantly increased after thyroidectomy. This elevation could not be explained. Injection of TRH, however, caused a rise of MOPEG concentration in all brain regions of thyroidectomised rats similar to that occur-

TABLE 1 Effect of TRH and T_3 , injected intraperitoneally, on the content of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) of whole brain and (or) various parts of rat brain

	Controls	Normal With TRH	Controls	With T_3	Thyroidectomised Controls	With TRH
Whole brain	121.3 \pm 6.0	156.1 \pm 9.0				
Cortex	105.2 \pm 3.1	126.4 \pm 4.2	93.5 \pm 2.2	103.1 \pm 2.5	98.3 \pm 2.5	120.8 \pm 2.7
Hypothalamus	182.7 \pm 11.0	226.3 \pm 8.3	150.8 \pm 5.8	167.6 \pm 5.6*	182.2 \pm 8.5	246.9 \pm 12.4
Mesencephalon plus medulla oblongata	115.3 \pm 4.9	143.5 \pm 6.0	117.0 \pm 3.9	127.0 \pm 3.7†	147.3 \pm 6.1	204.6 \pm 8.2
Rest of brain	103.0 \pm 2.0	128.0 \pm 5.5	105.0 \pm 3.9	128.0 \pm 4.3	119.3 \pm 4.6	175.4 \pm 6.4

Measurements were carried out 2 h after i.p. administration of 10 mg kg⁻¹ TRH or 20 μ g kg⁻¹ T_3 , respectively. Thyroidectomy was performed 2 d before injection of TRH. The figures indicate ng g⁻¹ and represent means with s.e.m. of values obtained from twelve to twenty-eight determinations per group performed in three to seven experiments. Each determination was carried out with one single whole brain or with pools of two to three brain parts.

Significance (compared with corresponding controls).

* $P < 0.05$.

† $P > 0.05$; all other values, $P < 0.01$ (Student's *t* test).

ring in non-operated animals (Table 1). T_3 (20 μ g kg⁻¹) administered intraperitoneally (i.p.) to normal rats caused a rise of MOPEG in cerebral cortex, hypothalamus and mesencephalon plus medulla oblongata which was less pronounced than that due to TRH. In the 'rest of the brain' a similar increase was found after T_3 and TRH administration.

Since TRH did not change rectal temperature, the TRH-induced increase of cerebral MOPEG was probably not due to hypothermia in which noradrenaline turnover is accelerated¹⁵. An inhibition of the clearance of MOPEG from the brain is also unlikely, since other amine metabolites (homovanillic acid and 5-hydroxyindoleacetic acid), which are thought to be cleared by a similar transport mechanism as MOPEG¹⁰, were not affected by TRH. Therefore, the TRH-induced increase of MOPEG in the brain is rather due to an enhanced release of noradrenaline with consecutive catabolism of the amine to the glycol. This is confirmed by histofluorimetric experiments. Thus, the α -methyl-*p*-tyrosine-induced diminution of the green fluorescence specific for catecholamines was accentuated by TRH in the hypothalamus and the cerebral cortex which mainly contain noradrenergic fibres (Constantinidis *et al.*, in preparation).

Intraperitoneal injection of 10 mg kg⁻¹ TRH 1 h before intraventricular administration of L-3-¹⁴C-tyrosine enhanced the accumulation of ¹⁴C-noradrenaline formed in the whole brain within 1 h (controls: 1,393 \pm 55 c.p.m. g⁻¹, TRH-treated: 1,600 \pm 62 c.p.m. g⁻¹; $P < 0.02$, $n = 25$). This indicates an increased synthesis of cerebral noradrenaline which probably compensated for its enhanced release since the cerebral content of the endogenous amine remained unchanged. On the other hand, TRH did not influence the accumulation of ¹⁴C-dopamine in the brain (controls: 4,291 \pm 198 c.p.m. g⁻¹; TRH: 4,433 \pm 296 c.p.m. g⁻¹; $P > 0.05$, $n = 15$) in agreement with the fact that the hormone has no effect on endogenous homovanillic acid (see above). These findings indicate that TRH enhances the turnover of noradrenaline in the rat brain. The potentiation by TRH of the behavioural changes caused by L-dopa plus pargyline in hypophysectomised mice¹⁷ might be connected with this effect. Since TRH also acted in thyroidectomised rats, a mediation by the hypophyseothyroid axis does not seem to be a major mechanism in the acceleration of noradrenaline turnover. Nevertheless, as indicated in the experiments with T_3 , a contributing role of this hormone in normal animals cannot be excluded.

In conclusion, TRH probably causes an activation of noradrenergic neurons in the brain. This effect might be connected with the antidepressant action of the tripeptide in man and if so, support the hypothesis of an involvement of noradrenaline in mental depressive states.

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Multiquantal release of acetylcholine in mammalian ganglia

IN the course of studies on synaptic transmission in the hypogastric ganglia of male guinea pigs it became apparent that in many ganglion cells repetitive stimulation of the hypogastric nerve at 1-Hz raised the frequency of spontaneous synaptic potentials from less than 1 min⁻¹ to about 1 s⁻¹. Many of these cells had only a single preganglionic fibre synapsing with them so that the spontaneous synaptic potentials arising from a single nerve could be readily observed. Blackman *et al.*¹ described large spontaneous synaptic potentials in this preparation and we decided to examine the amplitude distributions of spontaneous synaptic potentials

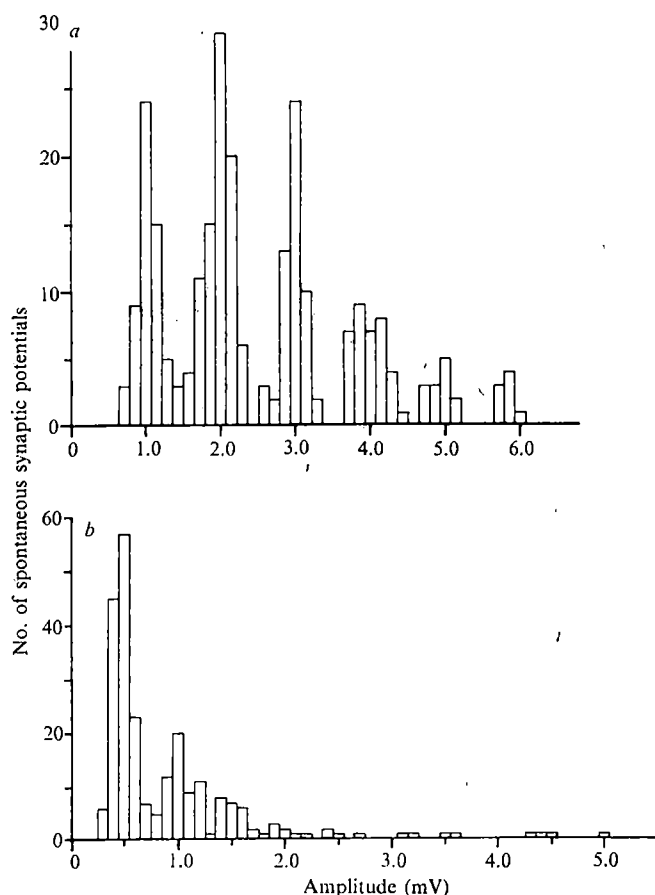


FIG. 1 Amplitude distributions of spontaneous synaptic potentials in *a*, a cell whose single input was repetitively stimulated at 1 Hz; *b*, a single input cell in 15 mM K^+ .

recorded during repetitive stimulation (1Hz). Although 'spontaneous' synaptic potentials which were not time locked to the stimulus occurred occasionally during the evoked response, only those recorded after the membrane potential had returned to normal were selected for analysis.

A typical amplitude distribution for the spontaneous synaptic potentials recorded from a cell during prolonged repetitive stimulation (at 1 Hz) of its single input can be seen in Fig. 1*a*. The most striking feature is the appearance of a number of distinct groups in the distribution; if each group was treated as a separate distribution, means of $1.1(\pm 0.02)$, $2.0(\pm 0.02)$, $3.0(\pm 0.02)$, $4.0(\pm 0.03)$, $4.9(\pm 0.04)$ and $5.8(\pm 0.03)$ mV were obtained. This phenomenon was observed in every cell (seven cells with a single input) from which spontaneous synaptic potentials were recorded during repetitive stimulation. To determine whether the observations were due to some effect of stimulation, another method of raising the frequency of spontaneous synaptic potentials was used.

Blackman *et al.*² found that increasing the K^+ concentration in the bathing medium markedly increased the frequency of spontaneous synaptic potentials recorded from frog sympathetic ganglion cells. In the present experiments it was found that increasing the K^+ concentration from 5 mM to 15 mM raised the frequency of spontaneous synaptic potentials to about $1 s^{-1}$ even if the hypogastric nerve was not stimulated. Thus a large sample of spontaneous synaptic potentials could be recorded in a relatively short time. If the hypogastric nerve was stimulated the number of preganglionic inputs on the cell could still be determined. Figure 1*b* shows the amplitude distribution obtained in 15 mM K^+ from a cell with a single input. Several distinct groups can be seen, although samples of larger amplitudes were too small to allow analysis. If the groups were treated as separate dis-

tributions the means obtained were $0.5(\pm 0.008)$ mV, $1.0(\pm 0.02)$ mV, $1.5(\pm 0.02)$ mV, $2.0(\pm 0.06)$ mV, $2.4(\pm 0.06)$ mV.

This preparation clearly differs from the rat diaphragm, where Hubbard and Jones⁸ found that the amplitude distribution of spontaneous endplate potentials, although often skewed to higher amplitudes, showed no evidence of grouping about multiples of the modal amplitude. They concluded that the 'giant' spontaneous endplate potentials first described by Liley^{4,5} were due to larger than normal quanta. The 'giant' spontaneous endplate potentials at the rat neuromuscular junction however, have a different time course to the miniature endplate potentials⁶ and their frequency is unaffected by raised K^+ concentration or repetitive stimulation⁵.

The spontaneous synaptic potentials recorded in the hypogastric ganglia fit a multimodal amplitude distribution. There are at least two possible explanations of these results. (1) The quanta (or packets) of acetylcholine (ACh) stored in the preganglionic nerve terminals may be of a number of different sizes; or (2) spontaneous release of ACh may occur in multiquantal units.

The first explanation implies that the contents of the larger quanta would be approximately integral multiples of the contents of the smallest quanta. If a vesicle represents a quantum and the concentration of ACh in the vesicle is approximately uniform throughout the population of vesicles then their diameters should conform with the series 1, 1.26, 1.44, 1.59, 1.71 and soon with the diameter of the smallest vesicle taken as 1 unit. Any attempt to find evidence for a distribution of vesicle diameters with peaks at these relative values would be difficult as such measurements would be complicated by the uneven sectioning of vesicles inevitable in electron-microscopy. A quantitative study of the diameter and density of vesicles in the nerve terminals in the hypogastric ganglia would be necessary to completely eliminate this hypothesis.

Multiquantal spontaneous synaptic potentials have been described in the chick ciliary ganglion⁷ and in the parasympathetic ganglion cells of the septum of frog heart⁸. Martin and Pilar⁷ proposed that the spontaneous release of a single quantum created a small but finite probability that a second quantum would be released virtually simultaneously, the 'drag' hypothesis. They were able to fit the amplitude distribution of spontaneous synaptic potentials to a Poisson distribution by calling a unit response a failure and a double response a single and calculating a 'quantal content' of the 'dragged', synaptic potential. In the present experiments, however, no good fit could be obtained to either a Poisson or a binomial distribution using the 'drag' hypothesis. Dennis *et al.*⁹ were able to fit their data with a model based on the availability of release sites; however, their analysis could not be applied to the present experiments as no evidence was available to the maximum number of quanta which could be spontaneously released.

The quantum contents of subthreshold evoked synaptic potentials in most cells of the superior cervical ganglion⁹ and the sympathetic chain ganglia¹⁰ of guinea pigs do not appear to fit a Poisson distribution. In these preparations large spontaneous synaptic potentials can be observed and it is possible that the mechanism responsible for the large spontaneous synaptic potentials is also involved in the evoked release. Large increases in the frequency of spontaneous synaptic potentials due to repetitive preganglionic stimulation have, so far, only been observed in cells with 'strong fibre' type input¹; such input always initiates an action potential obscuring the evoked synaptic potential. By raising the Mg^{2+} : Ca^{2+} ratio in the bathing solution the amount of ACh released by a single impulse can be reduced and the quantal content of individual synaptic potentials could then be measured. Altering the Mg^{2+} : Ca^{2+} ratio, however, may

change the amplitude distribution of the spontaneous synaptic potentials⁸ and an examination of the ionic basis of both the spontaneous and the evoked release mechanisms will be necessary to complete this analysis.

The occurrence of large spontaneous synaptic potentials during low frequency repetitive stimulation could be of significance in the function of autonomic ganglia. Most autonomic ganglia receive tonic synaptic input from the central nervous system, *in vivo*¹¹, or from neurones in the periphery¹² and the cells in these ganglia receive input from many preganglionic nerve fibres¹³⁻¹⁵. In most cells, summation of synaptic potentials is necessary to excite an action potential and so if spontaneous synaptic potentials occurred of comparable amplitude to the evoked synaptic potential these might well be a significant factor in determining the firing pattern of cells in these ganglia. Large spontaneous synaptic potentials have been observed in many mammalian ganglia receiving multiple inputs, for example, guinea pig sympathetic chain¹⁰, guinea pig superior cervical ganglion⁹ and guinea pig inferior mesenteric ganglion (J. C. Bornstein, unpublished). Thus the output of a ganglion cell may depend not only on activity in the preganglionic nerves but also on the frequency of the spontaneous synaptic potentials released by these nerves. This seems to depend on the past activity of the nerve terminal.

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Effects of β adrenergic blockade on plasma catecholamines in exercise

It is well established that physical exercise causes an increase in sympathetic activity, as shown by rises in the levels of plasma and urine catecholamines¹. This increase in endogenous adrenergic activity can be used to study the effects of sympathetic stimulation. Such studies usually compare a control situation with one in which either α or β adrenergic receptor activity is blocked pharmacologically, thereby allowing study of both the blocked and unblocked receptors. Beta blocking drugs have been used in this way to study changes in carbohydrate metabolism², free fatty acid metabolism³, and blood coagulation and fibrinolysis⁴ induced by exercise.

It has previously been assumed that because adrenergic blocking drugs act by blockade of the receptors, they do not affect the release of the catecholamines, and thus the magnitude of the sympathetic responses. A recent study⁵ has shown, however, that animals subjected to haemorrhagic shock under medication with α and β adrenergic blocking drugs in combination had lower levels of plasma catecholamines than the control animals in spite of an equivalent degree of blood loss and hypotension. Demonstration that single adrenergic receptor blockade also altered the extent of the sympathetic response in a stress situation would be of considerable importance, for in studies involving the use of

TABLE 1 Catecholamine concentrations

		Total catecholamine ($\mu\text{g l}^{-1}$)	Noradrenaline ($\mu\text{g l}^{-1}$)	Adrenaline ($\mu\text{g l}^{-1}$)
		Mean \pm s.e.)	Mean \pm s.e.)	Mean \pm s.e.)
Non medicated	Pre-exercise	0.66(± 0.13)	0.52(± 0.06)	0.14(± 0.16)
	Post exercise	1.80(± 0.32)	0.57(± 0.13)	0.23(± 0.26)
Propranolol	Pre-exercise	0.68(± 0.17)	0.52(± 0.08)	0.16(± 0.13)
	Post exercise	1.41(± 0.37)	0.78(± 0.21)	0.64(± 0.35)
Oxprenolol	Pre-exercise	0.50(± 0.09)	0.29(± 0.14)	0.21(± 0.13)
	Post exercise	1.69(± 0.55)	0.65(± 0.75)	1.04(± 0.55)
LB 46	Pre-exercise	0.78(± 0.20)	0.51(± 0.09)	0.27(± 0.16)
	Post exercise	1.69(± 1.11)	0.84(± 0.38)	0.85(± 0.73)

adrenergic receptor blocking drugs the unblocked receptor could be subjected to different degrees of adrenergic stimulation in the control and test situations. This would invalidate the current assumption that the extent of the sympathetic response is the same in both situations. This study reports the effect of three β adrenergic blocking drugs on the extent of the sympathetic response induced by exercise.

Five healthy males aged between 28 and 37 were studied at weekly intervals for four weeks. On each occasion the subjects had a light breakfast at 0800 h, and then reported to the laboratory at 1000 h. After resting on a couch for 15 min each was seated in a chair to allow removal of blood by venepuncture from an antecubital vein.

Each subject then exercised at 1,050 kilopond M min⁻¹ for 5 min. on a bicycle ergometer. On the first occasion the subjects exercised following the oral administration 2 h previously of 120 mg of oxprenolol (Trasicor, Ciba). In the second week, the experiment was repeated without medication and in the remaining two weeks the subjects were premedicated with 120 mg propranolol (Inderal ICI) and 12.5 mg Pindolol (LB46, Sandoz) respectively.

Immediately following exercise, blood was again taken from an antecubital vein with the subject in a sitting position. Blood samples were immediately put into 10 ml lithium heparin tubes, centrifuged and the red cells separated. The resulting plasma was frozen at -70°C and subsequently analysed by the trihydroxyindole fluorometric method for plasma catecholamine concentrations⁶. Measurements were made of total catecholamines and noradrenaline, the adrenaline level being deduced by difference. The plasma was also analysed for concentrations of the β blocking drugs to confirm adequate β blockade.

Comparison of the resting pre-exercise catecholamine concentrations in the medicated and non-medicated groups showed no difference in the noradrenaline levels. Adrenaline levels in the medicated groups were, however, marginally higher than those in the non-medicated group, but the dif-

ference was not statistically significant. Exercise in both groups caused rises in both plasma adrenaline and noradrenaline levels (Table 1). The extent of the response was however different in the β blocked and control groups. For example, whereas in the non-medicated controls noradrenaline

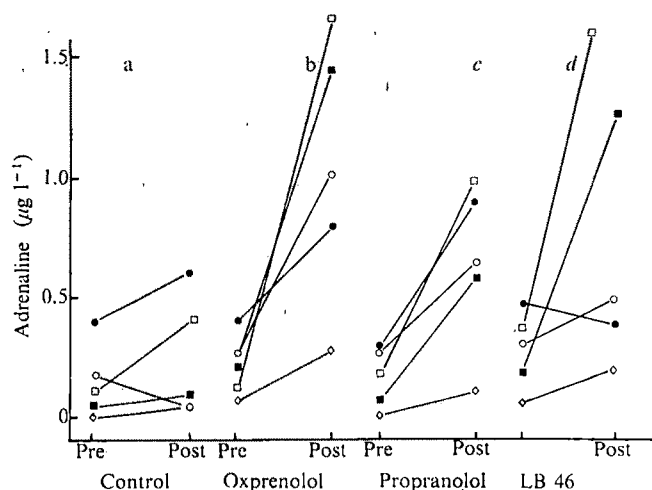


FIG. 1 Effects of adrenergic blockade on plasma adrenaline response to exercise. *a*, Control; *b*, oxprenolol; *c*, propranolol; *d*, LB 46.

rose from a mean of $0.52 (\pm 0.06)$ to $0.57 (\pm 0.13) \mu\text{g l}^{-1}$, and adrenaline rose from a mean of $0.14 (\pm 0.16)$ to $0.23 (\pm 0.26) \mu\text{g l}^{-1}$, the same workload in the group medicated with propranolol produced a rise of noradrenaline from $0.52 (\pm 0.08)$ to $0.78 (\pm 0.21) \mu\text{g l}^{-1}$ and a rise in adrenaline from $0.16 (\pm 0.13)$ to $0.64 (\pm 0.35) \mu\text{g l}^{-1}$. Subjects medicated with oxprenolol and pindolol showed similar results (Table 1, Fig. 1). The difference in the adrenaline response in the β blocked groups and the non-medicated control group was statistically significant ($P > 0.05$). Although the levels of noradrenaline exhibited a similar trend (Fig. 2), the values obtained in the medicated groups were not significantly different from those in the non-medicated group.

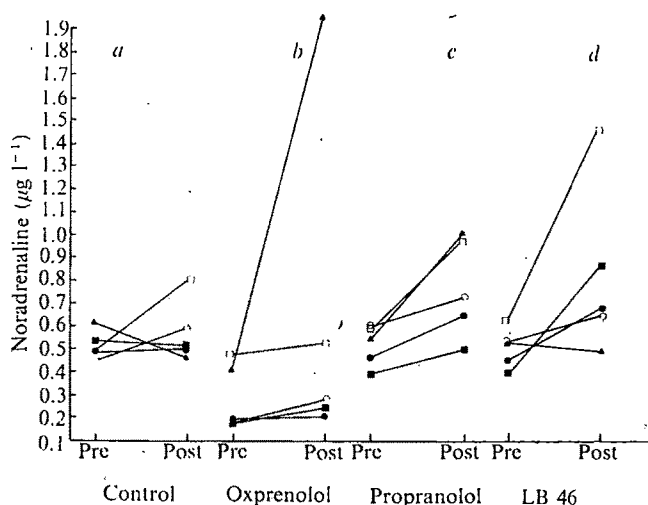


FIG. 2 Effects of adrenergic blockade on plasma noradrenaline response to exercise. *a*, Control; *b*, oxprenolol; *c*, propranolol; *d*, LB 46.

Evidence of adequate β blockade in the medicated groups was demonstrated both by a reduction in the degree of exercise-induced tachycardia evident in the nonmedicated group

and by measurement of the plasma levels of all three β blocking drugs.

Most of the volunteers found it more difficult to exercise when medicated with β blocking drugs, but in only one case was there any profound circulatory change. This was observed in one individual medicated with pindolol, who at the end of the period of exercise showed marked bradycardia, cutaneous vasoconstriction and syncope. It is interesting that his rises in adrenaline and noradrenaline (from 0.36 to $1.95 \mu\text{g l}^{-1}$ and from 0.63 to $1.47 \mu\text{g l}^{-1}$) were the greatest recorded in this study.

In all forms of exercise the sympathetic nervous system is activated to a greater or lesser degree. The stimuli for this sympathetic response include baroreceptor activation, pain and psychological factors. These results in the release of adrenaline and noradrenaline from the adrenal medulla and nerve endings respectively.

Von Euler⁷ considers that the increase in plasma catecholamines probably reflects compensatory adrenergic activity in non muscular vascular beds, designed to counteract the effects of the vasodilatation induced by exercise in the muscle beds. Activation of the vasomotor centre in exercise is *via* blood pressure homeostatic reflexes⁸. In addition, however, emotional stress occasioned by hard physical exercise could also contribute to the extent of the adrenergic response.

Our studies have demonstrated for the first time a significant difference in the extent of the adrenergic response to exercise in subjects under β adrenergic blockade. The exact reason for the excess adrenergic activity is not clear. The reliability of the fluorometric assay used is now established⁹ and the pre-exercise values in all four groups show that the β blocking drugs themselves have not interfered with the accuracy of the assay. This same observation also confirms that the results cannot be attributed to intrinsic adrenergic activity of the β blocking drugs. Account must be taken of the possible contribution of psychological stimuli to the increased adrenergic response under β blockade, for there is no doubt that in most cases, the volunteers found it more difficult to carry out the workload when medicated with β blockers. Anxiety in the pre-exercise period can, however, be excluded by examination of the resting catecholamine levels.

The most likely cause for the increased adrenergic response in the β -blocked individuals is an exaggeration of the compensatory response described by von Euler⁷. Beta blockade reduces the compensatory increase in cardiac output that normally occurs in exercise by 17% (ref. 10) and this, in association with dilatation in the muscle beds, can lead to a fall in arterial blood pressure¹¹ which activates an increased sympathetic response.

Irrespective of the cause of this increased adrenergic response to exercise under β blockade, the observation is of considerable significance for two reasons. First, it demonstrates that in experiments using exercise to stimulate sympathetic nervous activity to investigate its circulatory or metabolic effects, β adrenergic blockade alters the degree of the adrenergic response, and consequently the stimulation of the unblocked α receptor. Second, the information is of significance in the management of patients on treatment with β adrenergic blocking drugs. These drugs are widely used in the treatment of angina pectoris and hypertension. Our findings indicate that patients being so treated should be warned against undertaking sudden vigorous exercise, as this might result in an excessive adrenergic response which could have deleterious effects.

Beta adrenergic blockade with propranolol, oxyprenolol, or pindolol significantly increases the adrenergic response to exercise as judged by measurement of plasma catecholamine concentrations.

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Glutamate antagonists at a crayfish neuromuscular junction

As Kravitz *et al.*¹ have pointed out, glutamic acid is at present the only candidate for the excitatory transmitter at crustacean neuromuscular junctions. The evidence is that^{1,2} glutamic acid is the most potent excitatory substance present in the crustacean central nervous system (CNS), whole peripheral nerves and isolated excitatory axons^{1,3-5}; that the glutamic acid content in nerve extracts accounts for the whole of their excitatory activity on crustacean muscles¹, and that the receptors to glutamate are localised at the same junctional spots as the receptors to the natural excitatory transmitter⁶. Also, both glutamic acid and the natural transmitter produce similar changes in the permeability of the postjunctional muscle membrane⁷⁻⁹ and in spite of a large background leakage, a significant release of glutamic acid can be evoked by stimulation of excitatory nerves¹. There is also a selective uptake of glutamic acid at the neuromuscular junction which could provide an inactivation mechanism¹⁰. There is, however, no information on whether glutamate receptors have the same pharmacological properties as the receptors of the natural excitatory transmitter.

The main object of the work reported here has been to screen a series of compounds for a specific antagonist to glutamic acid. We have found some drugs which specifically block both the responses to iontophoretically applied glutamate and the excitatory junctional potentials (EJPs) recorded from the same crayfish muscle fibres.

We used the slow flexor abdominal muscle fibres¹¹ from the crayfish *Procambarus clarkii*. These muscles are located in the anterior wall of each abdominal segment, each side of the midline, just beneath the integument. One insertion is on a sternite and the other on an integumental ridge. The superficial layers of the anterior wall of an abdominal segment were removed together with both sternites limiting the segment and placed in a perspex chamber through which van Harreveld's solution was continuously circulated. The

integument covering the muscle was first excised to allow the transillumination of the muscles and the two sternites were then fixed to the bottom of the chamber, without stretching the muscle fibres, in order to expose the posterior face of the slow flexor muscles. Single fibres were impaled with two independent KCl-filled micropipettes separated by 100-200 μ m. One pipette was used for recording through an amplifier of unit gain connected to a 280 Brush pen recorder; the other was used to pass constant current pulses across the membrane for measuring the input resistance of the fibre and/or steady current for setting the membrane potentials to desired levels. Glutamate ions were applied iontophoretically on to the muscle fibres as an anion from a third micropipette filled with a 2 M monosodium glutamate solution at pH 8.0. When necessary, GABA was also applied iontophoretically as a cation, from another pipette filled with a 1 M GABA solution at pH 3.5.

To study the effect of the drugs on the EJPs advantage was taken of the fact that the slow flexor abdominal muscles fibres receive synaptic inputs from several 'spontaneously' firing motoneurons¹². A whole abdomen preparation was therefore set up in the chamber, the integument of one of the segments was removed and the fibres were impaled with a micropipette. In this preparation, the connections of the nerve cord with the muscles remained intact, and a barrage of 'spontaneous' EJPs could be recorded (Fig. 1). In all the experiments, the drugs tested were dissolved in the van Harreveld solution and perfused to the preparation.

When glutamate is applied to a slow flexor muscle fibre three main sensitive regions, separated by insensitive areas, are revealed: one at each end of the fibres near the insertions and a large field in the central parts of the fibre. The responses to glutamate consist of fast depolarisations (Fig. 2a),

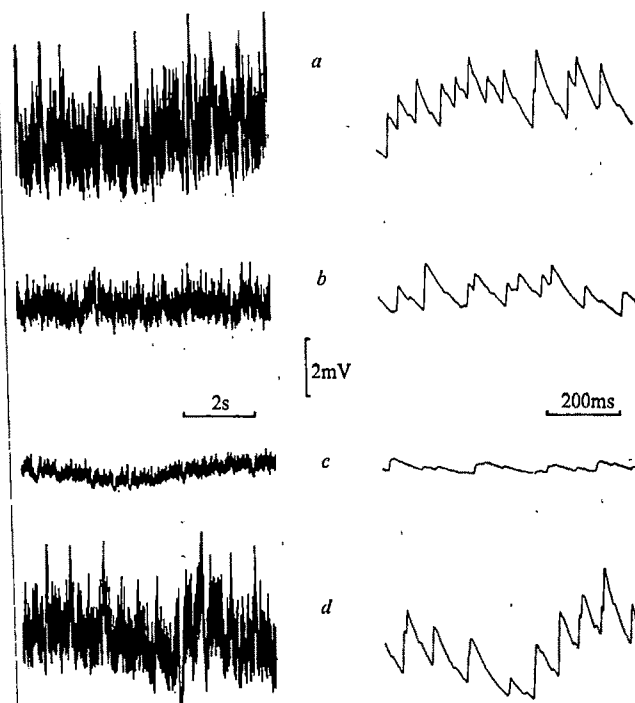


Fig. 1 Isolated crayfish abdomen preparation. The EJPs recorded from an abdominal slow flexor muscle fibre result from the 'spontaneous' firing of several motoneurons. In each horizontal row, the same synaptic activity was recorded at two different speeds. *a*, EJPs discharge when the preparation is bathed in a normal medium. *b*, After perfusing to the preparation a 10^{-3} M solution of L-glutamic acid γ -methylester, the amplitude of the EJPs is decreased. *c*, Decrease in the amplitude of the EJPs is almost complete after the concentration of the antagonist to 10^{-2} M. Notice in *b* and *c* that there is no evident alteration of the frequency of discharge of the EJPs. *d*, Recovery after prolonged washing.

easily desensitised by repeating the iontophoretic application, and showing the same time course and properties as the ones described by Takeuchi and Takeuchi⁶ in the dactyl muscles.

Of 56 drugs (glutamic acid structural analogues, enzyme inhibitors, drugs showing effects on other synapses), eight substances were found to block the glutamate responses (Table 1). The mechanism of their blocking action was analysed and their relative potency evaluated. The γ -methyl ester of L-glutamic acid (L-Glu- γ -ME) was the most potent of the drugs tested, followed by other methyl and ethyl esters of L-glutamic acid, piperazine, strychnine and phenylurea. The effect of these compounds on the input resistance of the muscle fibres was tested and only phenylurea and coumarin were found to increase consistently the resting membrane conductance. This effect was probably independent of the blocking effect since after these substances were

TABLE 1 Eight substances which block the glutamate response

Compound	Relative blocking efficacy	Effect on membrane conductance	Effect on GABA receptors
L-glutamic acid γ -methyl ester	100	No	No
L-glutamic acid dimethyl ester	90	No	No
L-glutamic acid monoethyl ester	60	No	No
L-glutamic acid diethyl ester	50	No	No
Piperazine	90	No	Blocks
Strychnine	80	No	No
Phenylurea	80	Increases	No
Coumarin	60	Increases	No

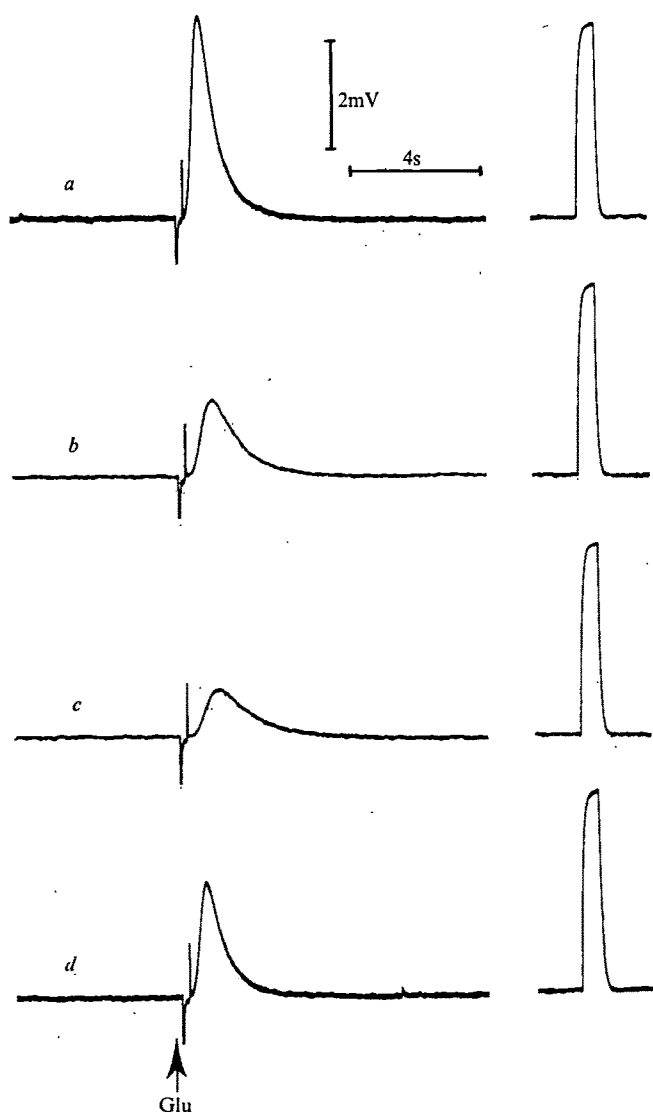


FIG. 2 A series of iontophoretic applications of glutamate on to an abdominal slow flexor muscle fibre of *Procambarus*. The vertical bars mark the duration of the application. At the right of the recordings, a series of electrotonic potentials obtained after each glutamate response by passing current pulses across the fibre membrane. *a*, Glutamate response and the electrotonic potential in a normal medium. *b*, Glutamate response decreases in amplitude after perfusion to the preparation of a 10^{-3} M solution of L-glutamic acid γ -methyl ester. *c*, Response to glutamate is almost blocked under action of a 2×10^{-3} M solution of the antagonist. In both *b* and *c* there is no change in the amplitude of the electrotonic potentials, that is, of the membrane resistance. *d*, Onset of recovery of the glutamate response after removal of the blocking agent.

removed the membrane input resistance rapidly recovered its control values, whereas the recovery of the glutamate responses was much slower. Because of these non-specific effects on the membrane conductance these two substances were unsuitable for analysing the synaptic inputs.

In addition, piperazine, which did not affect the muscle membrane conductance, blocked the responses not only to glutamate, but also to GABA. We considered that this made piperazine not worth further investigation. Finally, strychnine was found to be an effective antagonist of glutamate on these muscle fibres at concentrations of 10^{-3} to 10^{-2} M, without showing effects on the membrane conductance or the GABA responses. But since strychnine has been shown to block other receptors in other preparations (glycine receptors in vertebrate spinal neurones¹³ and acetylcholine receptors in molluscan neurones¹⁴) and to interfere with the release of transmitter in vertebrates¹⁵ and invertebrates¹⁶, we did not use it in the experiments on the EJPs.

The most suitable drugs for such analysis were therefore the esters of L-glutamic acid, in particular L-Glu- γ -ME. Figure 2(*b* and *c*) shows that at concentrations up to 2×10^{-3} M it blocks almost completely the response to glutamate without affecting the membrane conductance of the muscle fibre. Even at concentrations of 10^{-2} M no effect on the input resistance was observed. The recovery shown in Fig. 2*d* is only partial but after prolonged washing the effect was totally reversible. On the other hand, the same concentrations of antagonist did not affect the responses of the fibres to the iontophoretic application of GABA (Fig. 3*b*).

Haldeman and McLennan¹⁷ working with spinal and cuneate neurones found that the diethyl ester of L-glutamic acid was the most potent blocker of the series, whereas the methyl esters had agonistic effects. This reversed pattern suggests that the receptors in the vertebrate CNS and on crustacean neuromuscular junctions could be different.

The effects of L-Glu- γ -ME were assayed in the 'spontaneous' EJPs recorded in the slow flexor muscle fibres in an *in vitro* abdomen preparation (see above). In Fig. 1*a* a barrage of EJPs was recorded in these fibres at two different speeds. When L-Glu- γ -ME was applied to the preparation at concentrations of 10^{-3} M (Fig. 1*b*) and 10^{-2} M (Fig. 1*c*) the amplitude of the EJPs gradually decreased without evident alteration of their frequency. This effect was reversible (Fig. 1*d*) after prolonged washing.

These experiments show that L-Glu- γ -ME blocked both the responses to the iontophoretic application of glutamate and the EJPs recorded from the same muscle fibres, without altering the input resistance of the fibres, the frequency of the neuronal firing or the conduction velocity of the motor axons. Nevertheless, the possibility that this compound acts on the quantal content of the EJPs must be investigated in further experiments.

We conclude that in spite of its low affinity for the glutamate receptors, L-Glu- γ -ME is a highly specific antagonist of glutamate on the crayfish neuromuscular postjunctional mem-

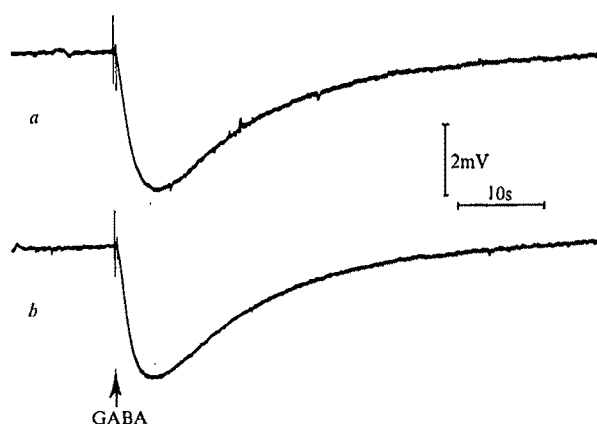


FIG. 3 L-glutamic acid γ -methyl ester does not affect the GABA responses of an abdominal slow flexor muscle fibre. *a*, Control hyperpolarising response to the iontophoretic application of GABA. *b*, GABA response remains unchanged after perfusing to the preparation a 5×10^{-3} M solution of the glutamate antagonist.

brane. The observation that it also blocks, very probably at the postsynaptic level, the generation of the EJPs is a new argument in favour of a transmitter role of glutamate in these neuromuscular junctions.

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Trichodermin resistance—mutation affecting eukaryotic ribosomes

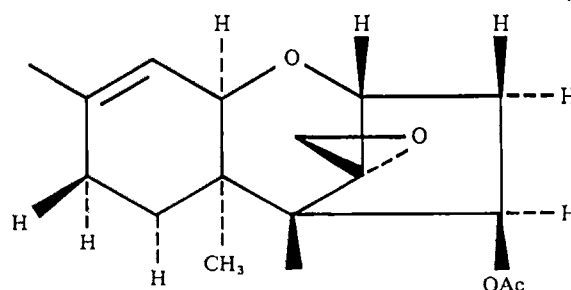
GENETIC studies of ribosomes in prokaryotes such as *Escherichia coli* have been facilitated by the use of mutants resistant to antibiotics; mutations which affect either subunit of the ribosome are known¹. In eukaryotes, genetic informa-

TABLE 1 Cross resistance between trichothecenes

Drug	CLP-1 (resistant) 100% = 13.2	A224A (sensitive) 100% = 63.1
Control		
Trichodermin (20 $\mu\text{g ml}^{-1}$)	100%	95%
T-2 Toxin (5 $\mu\text{g ml}^{-1}$)	67	26
T-2 Toxin (1 $\mu\text{g ml}^{-1}$)	82	53
Verrucaric A (5 $\mu\text{g ml}^{-1}$)	62	18
Verrucaric A (1 $\mu\text{g ml}^{-1}$)	69	27
Nivalenol (5 $\mu\text{g ml}^{-1}$)	99	40
Nivalenol (1 $\mu\text{g ml}^{-1}$)	95	55

S. cerevisiae strains A224A and CLP-1 were grown on a medium containing yeast extract-peptone-dextrose buffered to pH 5.8 with sodium succinate to about 3×10^7 cells ml^{-1} . The cells were collected, washed with ice-cold distilled water, resuspended in an equal volume of 100 mM Tris-50 mM KCl-5 mM Mg acetate-1 mM DTT (TKMD), and passed two times through a French pressure cell at 16,000–20,000 pound inch^{-2} . The suspension was centrifuged twice for 10 minutes at 18,000 r.p.m. in an SE-12 rotor (34 Kg). The top two-thirds of the supernatant was centrifuged for 2 h at 42,000 r.p.m. in a Ti50 rotor. The supernatant was removed avoiding the fluffy layer which was poured off and the pellet was resuspended in TKMD and 4 M NH_4Cl was added to a final concentration of 0.3 M and stirred for 30 min. The ribosomes were pelleted at 42,000 r.p.m. for 2 h, resuspended in TKMD and dialysed overnight against TKMD containing 0.6 mM β -mercaptoethanol. A supernatant fraction was prepared by precipitating the postribosomal supernatant with 70% ammonium sulphate and collecting the precipitate at 34,000 g for 20 min. The pellet was resuspended in one-third the original volume of TKMD and dialysed overnight in TKMD containing β -mercaptoethanol. Phenylalanine incorporation was performed in mixtures containing 50 mM Tris-HCl, pH 7.6, 100 mM KCl, 18 mM Mg acetate, 500 $\mu\text{g ml}^{-1}$ tRNA, 3.3 mg ml^{-1} creatine phosphate, 260 $\mu\text{g ml}^{-1}$ creatine kinase, 2 μM ^{14}C -phenylalanine ($10 \mu\text{Ci ml}^{-1}$), 1.0 mM ATP, 0.1 mM GTP and 500 $\mu\text{g ml}^{-1}$ poly (U). Incubations were carried out at 30°C for 30 min and terminated with 1 ml of 10% TCA. The reaction mixtures were filtered through GF/C glass fibre filters and washed five times with 3 ml of 5% TCA. Filters were dried and counted in a toluene-based scintillation fluor. Figures are expressed as percentage of control activity which is given in picomol of ^{14}C -phenylalanine incorporated.

tion on the translation apparatus has come mainly from studies of temperature-sensitive mutants^{2,10}, and also cycloheximide resistance, which is known to be a property of the 60S subunit³. Recent studies with resistance to the alkaloid cryptopleurine have shown that *Saccharomyces cerevisiae* strains resistant to this compound have altered ribosomes⁴.



Trichodermin

Trichodermin is a fungal toxin which belongs to the general class of 12, 13-epoxytrichothecenes; these compounds inhibit protein synthesis in eukaryotes but not prokaryotes (for a review, see ref. 5). The mode of action of these compounds on protein synthesis *in vivo* has been studied and the trichothecenes can be divided into two classes on this basis⁶. Some of the trichothecenes (such as verrucaric A and nivalenol) inhibit a step related to or shortly after initiation, while trichodermin affects elongation and/or termination by interfering with the peptidyl transferase reaction on the ribosomes. We now report the isolation of a spontaneous mutant of *S*

TABLE 2 Localisation of resistance to nivalenol on *S. cerevisiae* ribosomes

(a)	Ribosomes	Supernatant	Control	+ Nivalenol (5 μ g ml ⁻¹)	% Activity
	A224A	A224A	82.4	19.0	23
	A224A	CLP-1	51.5	17.0	33
	CLP-1	A224A	33.5	32.8	98
	CLP-1	CLP-1	8.9	8.7	98
(b)	Subunit source				
	40S	60S			
	A224A	A224A	69.8	22.3	32
	CLP-1	CLP-1	33.7	34.4	102
	CLP-1	A224A	84.2	23.6	28
	A224A	CLP-1	31.2	31.2	100
	A224A	—	1	—	—
	—	A224A	4.2	—	—
	CLP-1	—	3.9	—	—
	—	CLP-1	6.0	—	—

Ribosomal and supernatant fractions were prepared as described in the legend to Table 1. Free ribosomes were prepared as described by van der Zeijst *et al.*⁹ and subunits were separated by centrifugation through a 10–30% sucrose gradient in 50 mM Tris-HCl, pH 7.6 containing 500 mM KCl and 5 mM Mg acetate for 7 h at 22,500 r.p.m. in a SW-27 rotor at 15° C. The supernatant fraction used with the subunits was derived from the wild-type sensitive strain A224A. 0.13–0.20 A 260 units of each subunit was used in reaction. The incorporation of phenylalanine was assayed as in Table 1. Activity is expressed as total picomol of ¹⁴C-phenylalanine incorporated. An independently isolated mutant, CLP-8, gave similar results.

cerevisiae A224A which is resistant to 20 μ g ml⁻¹ of trichodermin and show that this mutant has an altered 60S subunit.

Ribosomes and supernatant fractions from a strain sensitive to trichodermin, A224A, and a strain resistant to trichodermin CLP-1, were prepared and tested for their response to trichodermin and related trichothecenes in Poly(U)-directed polyphenylalanine synthesis. Ribosomes from the sensitive strain were strongly inhibited by several of the trichothecenes (Table 1). In agreement with previous reports⁷, trichodermin was not an effective inhibitor of the poly(U) system. Polypeptide synthesis in extracts of the trichodermin-resistant strain was markedly resistant to nivalenol, and since trichodermin is not an effective inhibitor in the poly (U) system, we have used nivalenol to determine which component of the protein-synthesising system is altered in the strain resistant to trichodermin.

Table 2 shows that resistance to nivalenol in *S. cerevisiae* CLP-1 was associated with the ribosomes; specifically with the 60S subunit. As a further demonstration that trichodermin resistance was associated with the ribosomal fraction of strain CLP-1, we tested the effect of trichodermin on

puromycin-induced release of nascent peptide chains on polyribosomes (puromycin reaction). In the sensitive strain the puromycin reaction was inhibited in the presence of trichodermin, whereas the puromycin reaction on polyribosomes of *S. cerevisiae* CLP-1 was substantially resistant to trichodermin (Table 3).

As well as providing important genetic information on the structural components of ribosomes, antibiotic-resistant mutants also provide clues to the mode of action of an inhibitor. The demonstration of trichodermin resistance as a property of the 60S subunit of resistant strains is consistent with the notion that trichodermin inhibits the peptidyl transferase reaction (a property of the 60S subunit⁸). It has been suggested previously that it could act by preventing access of the aminoacyl-tRNA acceptor to the peptidyl transferase centre⁷.

Genetic studies have shown *S. cerevisiae* to have at least 17 chromosomes. Cycloheximide resistance, *cyh2*, a property of the 60S subunit, maps on chromosome VII² and cryptopleurine resistance, *cry1* (another ribosomal alteration) maps on chromosome III (ref. 4 and P. Grant, unpublished observations). Genetic analysis of CLP-1 indicates that trichodermin resistance is not linked to *cyh2* or *cry1*. Although there is no evidence that these mutations affect ribosomal proteins and not rRNA or modifying enzymes, the organisation of structural genes for ribosomes in simple eukaryotes such as *S. cerevisiae* may prove to be different from the ribosome gene 'cluster' found in prokaryotes such as *E. coli*. Recently, the genes for ribosomal proteins of *Drosophila* have been mapped and all ribosomal protein genes may be located within a 'cluster' in this eukaryote¹². In addition, the *Drosophila* ribosomal protein genes are linked to rRNA genes. In *S. cerevisiae* it has been shown that 70% of the rRNA is coded for by chromosome 1 (ref. 13). Trichodermin resistance is not linked to *adel* on this chromosome; therefore none of the three mutations, cycloheximide resistance, cryptopleurine resistance, or trichodermin resistance, which are known to affect ribosomes in *S. cerevisiae* are linked to the known rRNA genes or to each other.

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TABLE 3 ³H-Puromycin-induced release of polypeptide chains from yeast polyribosomes

	– Trichodermin	+ Trichodermin	% Inhibition
A224A			
Polysomes	2.2, 2.5	0.7, 0.6	67.3
CLP-1			
Polysomes	3.2, 1.1	2.3, 0.7	30.9

Yeast spheroplasts were prepared as described by Hartwell and McLaughlin¹⁰, substituting 1 M sorbitol for the 0.4 M MgSO₄. They were collected by centrifugation, resuspended in TKM and lysed with 0.5% sodium deoxycholate and 1% Brij 58 (Atlas Chem. Co.). The lysate was layered on a discontinuous gradient of 20% sucrose–60% sucrose in TKM, and centrifuged at 35,000 r.p.m. for 4 h in the Ti50 rotor. The pellet was resuspended in TKM. Assays were performed as described by Pestka *et al.*¹¹ except that GF/C glass fibre filters were used to collect the insoluble peptidyl-puromycin fragments. Trichodermin was present as indicated at 20 μ g ml⁻¹. Reactions were incubated at 30° C for 5 min. Activity is expressed as total picomol of ³H-puromycin incorporated into peptidyl-puromycin.

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book reviews

Animal interactions

Models in Ecology. By J. Maynard Smith. Pp. xii+146. (Cambridge: London, January 1974.) £3.70; \$10.50.

Ecology suffers from a surfeit of fascinating, but apparently unrelated observations, superimposed upon an acute shortage of general theories. An inevitable consequence has been the growing importance of general, precise, rather simple models which may provide insights into the workings of the real world, without its baffling complexity. This book provides a particularly good example of the approach, although it is by no means a general account of the ways in which models have been, and are being used in ecology. The problems which it chooses to examine range from predator-prey systems (chapters 2, 4, 6 and part of 11), the importance of time delays in population dynamics (chapter 3), competition (chapters 5 and 9), stability and complexity (chapters 7, 8, 10 and 11) and territorial behaviour (12), with the underlying theme throughout being the problem of stability in ecological systems. Virtually all the models are in the form of deterministic differential equations, the justification for using deterministic models being discussed briefly in chapter 1. In a comparatively small number of instances difference equations are used, while Leslie matrices are mentioned so briefly in chapter 3 that their inclusion hardly seems worthwhile.

As Maynard Smith points out, "the purpose of mathematics is to render the assumptions lying behind the arguments more explicit". But that is only the first step, albeit an important one. Conclusions derived through use of mathematics, and hence the assumptions themselves, should obviously be tested repeatedly against the real world, and even more importantly the models should suggest new insights and suggest new observations that are worth making. There are a disappointingly few places in this book where really new insights emerge, although it provides additional information and very useful summaries of a number of previously fairly well worked ideas. Material that is substantially new is to be found in chapter 6, which considers the effect of migration between sub-populations on the stability of predator-prey systems. It is a great pity that the logic used in setting up the model (pages 75-83) is not more fully explained. It seems to contain some cu-

rious assumptions, including the one that the addition of predators will make the prey in a subpopulation reach a larger population size faster than would be the case in the absence of predators. This does not seem reasonable. It is also a pity that no attempt has been made to find an analytical solution to the migration models, at least for the case of the simple model.

Chapter 10 includes a new analysis of a simple food web which attempts to provide a partial answer to the important question of how the number, and strengths, of the connections may influence the stability of the component populations. Again, an analytical solution is not attempted (and in this case one may not exist), but a simulation provides some interesting conclusions. Finally, chapter 12 provides a particularly good example of how an extremely simple model (in this case concerned with the influence of territorial behaviour on population density in birds) can provide some very interesting new insights into a well studied, but little understood, phenomenon.

In a number of other places in the book, the analysis may not be particularly new, but what it does do is explain well the main features of, or drawbacks and advantages in the types of analysis used by other workers. For example, it has some interesting comments to make about the 'standard' competition equations, and it provides an excellent summary of the major drawbacks inherent in the application of statistical mechanics to community modelling. Despite the sophistication of the mathematics, efforts in this direction would seem to be totally misdirected.

One of the main problems with the book is the patchiness with which it makes successful contact with the real world. Indeed this is partially acknowledged by the author, and by itself is not a major criticism, unless the reader (in making the contact for himself) finds a number of the models inadequate. Thus, there are rather sweeping generalisations being made about predator-prey systems in general, that are based almost entirely on models of predators which feed on only one species of prey, without questioning whether this is actually typical of most real predators. The fact that it isn't is never mentioned.

Nor are some of the actual attempts to relate observations and models equally successful. Some, like the analysis of Nicholson's blowflies in chapter 3 seem

to be good, but others, like the attempts to relate the stability properties of community models to Elton's and Watt's observations seem to lose sight of the obvious point that Elton and Watt are largely talking about population fluctuations, and the models are dealing with neighbourhood stability. These are not necessarily the same thing. Until ecologists build community models which consider global stability, and until they have experimental data that show whether the fluctuations one observes in nature are really unstable, or whether they merely represent, say stable limit cycles, movements towards equilibria with long damping times and continual disturbance, or what have you, most of the elegant discussion on pages 112-5 is not relevant.

It is certainly not a book for someone without a fairly good grounding in ecology. Equally it is not particularly suitable for someone who wants to learn how to apply certain techniques. One could not, for example, find out how to go about solving the general neighbourhood stability properties of a differential equation model. What it does do is show how apparently complex ecological problems can often be approached by rather simple models, and how such models can be used to 'get the feel' of the problem. If it encourages more practicing ecologists to do this it will have served a useful purpose.

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Mathematical magnetism

An Introduction to Electromagnetic Theory. By P. C. Clemmow. Pp. xi297. (Cambridge University: London, 1973.) \$10.

IN explaining his reasons for adding another text to the many which already deal with electromagnetism at undergraduate level, Dr Clemmow makes the point that competition from other topics in a widening curriculum leaves less time than formerly for a student to master the fundamentals of electromagnetism and this calls for a more crisp approach than has been usual. This idea determines both his choice of material and the manner of presentation. The material is pared down to theoretical essentials, very little being allowed in purely to illustrate or for reinforcement, while the style is disciplined and economical, making each point once only, and that in a rather austere mathematical way. Within this framework,

the route through the theory is not unconventional. Vector algebra and MKS units are used throughout and Maxwell's equations introduced fairly soon, to be followed by chapters dealing with electrostatics, magnetostatics, waves and properties of media. Each chapter is followed by a set of problems, mostly of the 'show that' variety—otherwise no answers are provided.

It is consistent with the author's aim that there are few concessions to any weakness in the reader. It is assumed that he is fully conversant with vector algebra and needs little assistance in visualising the significance of the equations. Magnetic field lines for example are mentioned first in Chapter 4 and so are not employed in the fairly lengthy discussion leading to Maxwell's equations in chapter 2. The approach of this discussion is again mathematical as opposed to physical. For instance, the equation $\text{div } B = 0$ appears as a consequence of $B = \text{curl } A$ rather than as a statement of the absence in nature of magnetic poles.

It is difficult to judge how big is the gap which the book is designed to fill. Well prepared students will appreciate the lack of distractions and the numerous mathematical points succinctly made. It would hardly be appropriate though for those, perhaps more typical students, who find they need to learn

mathematical methods and physical theory together and also need to surface frequently from abstract depths to reinforce new ideas with physical pictures and test them in the context of familiar practical applications.

S. CLOUGH

Raman spectra

The Raman Effect. Vol. 2: Applications. Edited by A. Anderson. Pp. xi+405-1033. (Dekker: New York, July 1973.) \$45.

THE initial impact of this book may well be governed by its price since \$45 is a particularly excessive sum to pay for a textbook. But on closer acquaintance it is seen to be rather less the extortion of funds from university libraries than it might appear since the book consists of two or three sub-books in the form of reviews, 100-200 pages in length, which are both authoritative and comprehensive.

The first chapter by Tobias gives applications of Raman spectroscopy to inorganic chemistry, describing experimental techniques and how to interpret results to obtain structural information. There are also sections on solution equilibria and fused salts and it provides a most valuable review. It is followed by a chapter on electronic Raman transitions from Koningstein and Mortensen. This is a very concise account, which contains the essentials of the use of the technique in identifying low-lying electronic states in crystals containing rare-earth ions. The next chapter is a treatise by Weber on high resolution Raman spectra of gases. This very complete account discusses the theory of rotational Raman, and its use in molecular structure determination. It also gives useful information on experimental techniques in detecting the weak signals characteristic of gas phase work.

Finally, there are two chapters on the spectra of crystals. A short introduction to the study of molecular crystals is given by Savoie who outlines the theoretical basis of the treatment of pure solids. This chapter mainly concerns crystals of simple molecules such as HCN and CF₄. The last chapter by Wilkinson is an outstanding review of the spectra of ionic, covalent and metallic crystals. Theoretical principles are lucidly explained in the early part of the chapter and the author then discusses assignments and experimental data for a large number of crystalline systems classified into different types. In each case the analysis is presented together with a discussion of available data. In addition there is a section on scattering from Landau levels, magnons and states other than phonons.

This collection of very authoritative articles will provide an invaluable refer-

ence text for workers in the field of Raman spectroscopy. The price of the book will ensure that few individuals will purchase it. This is particularly emphasised since this volume does not contain a chapter devoted to the general theory of the Raman effect and thus its companion volume is required. None the less it is a well produced book and it fills several gaps in the literature with authority.

A. J. McCaffery

Master class

Molecular Techniques and Approaches in Developmental Biology. Edited by Maarten J. Chrispeels. Pp. xii+306. (Wiley-Interscience: New York and London, October 1973.) £8.25.

THE mixed bag of articles making up this book originated in the La Jolla Summer Workshops on Molecular Techniques in Developmental Biology. Each chapter is based on a practical project run by an invited scientist, to teach the techniques used in his own research. The net result is a book which lacks coherence and covers only a limited amount of ground but, nevertheless, contains an enormous amount of very practical information, often in the form of step by step instructions with hints on how to adapt the method to other tissues. Obviously such a book can never be a real substitute for learning at the bench of a master and absorbing his or her approach towards developmental problems, but in the absence of opportunities for such personal contact it is certainly the next best thing.

The more prosaic of the eleven chapters deal with the fractionation of sub-cellular organelles, the separation of isozymes by gel electrophoresis and proteins by gel filtration, and the isolation of DNA from eukaryotic cells. Other chapters provide very detailed accounts of how to measure the number of progesterone receptor sites in rat uterus, how to calculate the real rate of RNA synthesis in sea urchin embryos incubated with ³H-adenosine by measuring the specific radioactivity of the ATP pool, and how to study changes in the population of transfer RNA species in developing tissues. The chapters I found most interesting were those by Pong and Loomis on the isolation of RNA polymerases from *Dictyostelium*, and by Bob Church on the theory and methodology of DNA-DNA and DNA-RNA hybridisation as applied to nucleic acids from cells of higher animals. The latter is a particularly lucid description of some very complex techniques and covers a lot of ground, including the increasingly important method of RNA-DNA hybridisation in a vast excess of DNA.

BRIGID HOGAN

THE HISTORY OF QUANTUM THEORY

Friedrich Hund

Professor at Göttingen University

Translated by Gordon Reece
M.Sc., Imperial College, London

Professor Hund — one of the few remaining scientists with a first-hand knowledge of the development of quantum theory in physics and chemistry between 1900 and 1927 — describes comprehensively and accurately the major discoveries, ideas and the people in this exciting period of scientific history.

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Books



matters arising

Waste and the Pacific

SIR,—The letter which appeared in *Nature* concerning the distribution of tar along latitude 35°N in the Pacific Ocean¹ raises the question of the cause of the pronounced peaks which occur.

One of the explanations advanced is that meanders in the Kuroshio current could be responsible for the distribution. If this were the case, the distribution would have peaks corresponding to the meander wavelengths, which are typically 200 km to 400 km (2° to 4° longitude). This is unlikely to be correct, since the three pronounced peaks are separated by distances of 2,000 km (20° longitude) and 1,300 km (13° longitude).

Another possible explanation of this distribution is that the intense Kuroshio current may generate a series of Rossby waves, which can propagate across the entire Pacific Basin².

In a numerical model recently developed by myself (to be published) a surface layer, 100 m deep and in an idealised basin, was subjected to a zonal

wind stress typical of winter in the Northern hemisphere. The energy dissipation was parameterised to occur

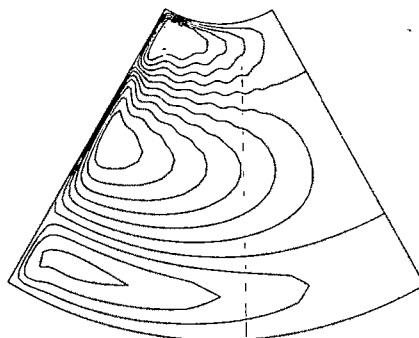


FIG. 1 The pattern of streamlines induced by a steady wind field in a hypothetical ocean basin, which extends from the equator to 60°N, and has a width of 60° longitude. The flow of the water is parallel to the streamlines and its intensity is proportional to the concentration of streamlines. The intense flow on the western edge of the basin is representative of the Kuroshio current in the North Pacific and the Gulf Stream in the North Atlantic.

over the continental shelves. The resulting steady circulation produced (Fig. 1) shows 'ripples' in the eastward flowing current between the subarctic gyre and the sub-tropical gyre (30°N to 55°N). The wavelengths in this particular example decrease from 1,300 km (13° longitude) in the western half of the basin to 600 km (6° longitude) in the east. The amplitude of meridional velocity at 35°N decreased from 8 cm s⁻¹ in the west to 4 cm s⁻¹ in the east.

I suggest, therefore, that the observed distribution of tar may be explained more satisfactorily in terms of the type of waves predicted by the model.

Yours faithfully,

N. C. WELLS

Department of Geophysics,
University of Reading,
Reading RG6 2AO

¹ Wong, C. S., Green, D. R., and Cretney, W. J., *Nature*, **247**, 30 (1974).

² Moore, D. W., *Deep Sea Research*, **10**, 735 (1963).

obituary

G. P. Kuiper

GERARD PETER KUIPER died suddenly on December 24, 1973, after a long and greatly distinguished career of ardent devotion to astronomy. He has left his mark by his achievements in observational and theoretical research and in developing new instruments, and in many other ways—as a director and founder of research institutes, as a writer and editor in the grand manner, as from the outset a prominent collaborator in the NASA lunar and planetary programmes which he called “the greatest scientific venture of history.”

Kuiper was born on December 7, 1905 in the Netherlands. In 1933 he gained his doctorate in Leiden for a thesis on binary stars with the renowned Ejnar Hertzsprung as his adviser. He then went to work mainly on the same subject at Yerkes Observatory, where Otto Struve had assembled perhaps the most brilliant

team of young astronomers in existence. After spending the year 1935–6 at Harvard, Kuiper returned to Yerkes as a staff-member and, apart from civilian scientific war services—he had become a United States citizen in 1937—he remained until 1960, twice serving as Director of the Yerkes and McDonald Observatories 1947–49, 1957–60. He then moved to the University of Arizona in Tucson, where he founded and directed the Lunar and Planetary Laboratory and its Catalina Observatory with an armoury of large telescopes for planetary work. These included the well-known 61-inch high resolution telescope specially adapted for observations in the near infrared, one of the many fields in which Kuiper was a pioneer. He relinquished some administrative responsibilities in 1973, but at the time of his death he was planning fresh developments of his scientific work. In the United States and abroad, Kuiper received numerous hon-

ours for his contributions to astronomy; the Royal Astronomical Society elected him an Associate in 1951.

As long ago as 1937, Kuiper's paper on Hertzsprung-Russell diagrams of stellar clusters pointed the way to much subsequent empirical work on stellar evolution. His pioneering work with Stromgren and Struve on models for close binary stars initiated what is still one of the most lively branches of stellar astrophysics. His classical papers of 1938 on the empirical mass-luminosity relation and of 1942 on “the nearest stars” remained for over a decade the main compendia of empirical stellar parameters.

Kuiper's dominating lifelong interest was, however, the solar system. He discovered two new satellites Miranda (Uranus) and Nereid (Neptune), the atmosphere on Saturn's satellite Titan, and the asteroid that bears his name. From 1960 onwards he produced (with

collaborators) four successive atlases of the Moon, based first on observations from Earth and then from space vehicles, and these played a vital role in choosing sites for Apollo landings. All this effort was inspired by the hope of ultimately elucidating the origin of the solar system.

With the astronomer Barbara M. Middlehurst, Kuiper edited the two famous series *The Solar System* (four of its five volumes are published), and *Stars and Stellar Systems* (seven of its nine volumes are published). Indeed, astronomers have seemed to be more excited when a new Middlehurst-Kuiper volume appeared than they were when quasars or pulsars were discovered.

Announcements

Appointments

The Council of the University of Bristol has appointed D. C. Smith, of the Department of Agricultural Science of the University of Oxford, to the Melville Wills Chair of Botany.

Erratum

In the article "Fluctuations in oil flow before and after earthquakes" by E. Arieh and A. M. Merzer (*Nature*, 247, 534; 1974) the former author is at the Seismological Laboratory, Geological Survey of Israel, Jerusalem and the latter at the Department of Environmental Sciences, University of Tel Aviv.

International Meetings

April 15-19, **9th International Symposium on Remote Sensing of the Environment** (Extension Service, Conference Department, The University of Michigan, Ann Arbor, Michigan 48104)

April 16-17, **The Application of Chemical Engineering to the Treatment of Sewage and Industrial Liquid Effluents** (Dr D. Geldart, Postgraduate School of Studies in Chemical Engineering, University of Bradford, Yorkshire)

April 16-18, **Optical and Acoustical Microelectronics** (Jerome Fox, Polytechnic Institute of Brooklyn, MRI Symposium Committee, 333 Jay Street, Brooklyn, New York 11201)

April 16-18, **University of Dacca Physics Symposium** (Professor A. M. Harunar Rashid, Organising Secretary,

Physics Department, University of Dacca, Dacca 2, Bangladesh)

April 17-19, **2nd Conference on Negative Ions** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

April 18-19, **Annual Congress and Scientific Exhibition of the British Institute of Radiology** (The General Secretary, The British Institute of Radiology, 32, Welbeck Street, London W1M 7PG)

April 22-24, **1st International Conference of the Aslib Transport and Planning Group** (Mr C. C. Parker, The Library, University of Southampton, Southampton SO9 5NH, UK)

April 22-25, **Joint American Physical Society and Optical Society of America Meeting** (The American Institute of Physics, 335 East 45 Street, New York 10017)

April 22-26, **Biochemische Analytik 74** combined with **1st European Congress of Clinical Chemistry** (Secretary General, Dr Rosmarie Vogel, D-8000 Munchen 2, Nussbaumstrasse 20, West Germany)

April 22-26, **Conference on Anomalous Scattering** (S. C. Abrahams, (International Union of Crystallography), Bell Laboratories, Murray Hill, New Jersey 07974)

April 22-26, **The Engineer in Society (EUROCON '74, c/o Klvl, 23 Prinsessegracht, The Hague, The Netherlands)**

April 22-26, **European Conference on Electrotechnics** (Mr G. Gaikhorst, c/o FME, Nassaulaan 13, The Hague, The Netherlands)

April 22-May 3, **Exploitation of Seismograph Networks** (International Advanced Study Institute, Sandefjord, Norway)

April 24, **Easter Lecture for Young People: Instant Energy-Heat Release with a Bang** (L. F. Linnett, Lecture Secretary, The Institute of Fuel, St Bernards House, St Bernards Road, Tonbridge, Kent TN10 3NL)

April 24-25, **Conference on Rheology of Clays and Cement Pastes** (Dr G. H. Tattersall, Department of Building Science, University of Sheffield, Arts Tower, Sheffield S10 2TN)

April 28-May 2, **76th Annual Meeting and Exposition of the American Ceramic Society** (Dr Peter Hawkins, Program Chairman, California Portland Cement Company, Box 947, Colton California 92324)

April 30-May 2, **3rd International Cannabis Conference** (The Conference Secretary, Institute for the Study of Drug Dependence, Chandos House, 2, Queen Anne Street, London W1M 0BR)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Council for Academic Freedom and Democracy. Annual Report 1972-1973. Pp. 16. (London: Council for Academic Freedom and Democracy, 1973.) [151]
 Meteorological Office. Geophysical Memoirs No. 119: A Climatology of the Stratosphere Over North-West Europe. By R. A. Hamilton, B. D. Mason and G. C. Bridge. (Met.O.864a.) Pp. 37. (London: HMSO, 1973.) £2.10 net. [161]
 Arthritis: a Vitamin Deficiency Disease. By Dr E. C. Barton-Wright. Pp. 32. (London: United Trade Press Ltd, 1973.) £1.25. [412]
 The Piltown Man Hoax. (Palaeontology Leaflet No. 2.) Pp. 7. (London: British Museum (Natural History), 1973.) 7p. [412]
 Patterns of Growth. By Jerome S. Bruner. (Inaugural Lecture delivered before the University of Oxford on 25 May 1973.) Pp. 22. (Oxford: Clarendon Press, 1974.) 50p net. [612]
 The Medical Research Council of Ireland. Annual Report for the year ended December 31, 1972. Pp. 106. (Dublin: Medical Research Council of Ireland, 1973.) 25p. [612]
 British Antarctic Survey. Scientific Reports, No. 67: The Geology of Parts of the Bowman and Wilkins Coasts, Antarctic Peninsula. By A. G. Fraser and P. H. Grimley. Pp. 59+8 plates. (London: British Antarctic Survey, 1972.) £2.80 net. [712]

Other Countries

An Annotated Bibliography, 1797-1969. By Warren Addicott. Pp. iii+201. \$1.50. Bulletin 1374: Placer Deposits of Alaska. By Edward H. Cobb. Pp. vi+231+plate 1. \$3.10. Water-Supply Paper 2019-B: Generalization of Stream-Temperature Data in Washington. By M. R. Collings. Pp. iv+45. 30 cents. Water-Supply Paper 2092: Quality of Surface Waters of the United States, 1968. Part 2: South Atlantic Slope and Eastern Gulf of Mexico Basins. Pp. x+373. \$2.35. Professional Paper 526-C: Cretaceous and Early Tertiary Depositional and Tectonic History of the Livingstone Area, Southwestern Montana. By Albert E. Roberts. Pp. iv+120+plates 1-3. Professional Paper 306-E: Geology and Paleontology of Canal Zone and Adjoining Parts of Panama. Description of Tertiary Mollusks (Additions to Gastropods, Scaphopods, Pelecypods—Nuculidae to Malleidae). By W. P. Woodring. Pp. iii+453-539+plates 67-82. \$1.75. (Washington, DC: Government Printing Office, 1973.) [3010]
 Animal Models of Human Disease: a Handbook. Pp. 98. (Reprints.) (Washington, DC: The Registry of Comparative Pathology, Armed Forces Institute of Pathology, 1973.) [3110]
 Smithsonian Contributions to Zoology. No. 120: A Systematic Monograph of New World Ethmid Moths (Lepidoptera: Gelechioidea). By Jerry A. Powell. Pp. iv+302. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) \$3.85. [3110]
 Nederlandse Vereniging voor Weer- en Sterrenkunde. Observations of Variable Stars, January-June 1973. (Report No. 24.) Pp. 7. (Groningen, Netherlands: Kapteyn Astronomical Laboratory, 1973.) [3110]
 Smithsonian Contributions to the Earth Sciences, No. 10: Mineralogy, Mineral-Chemistry, and Composition of the Murchison (C2) Meteorite. By Louis H. Fuchs, Edward Olsen and Kenneth J. Jensen. Pp. iii+39. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office, 1973.) 75 cents. [611]
 Ecology and Resource Development in Southeast Asia: a Report to the Ford Foundation. By Gordon Conway and Jeff Romm. Pp. 82. (New York: Ford Foundation, 320 East 43 Street, 1973.) [711]
 Smithsonian Contributions to Zoology. No. 150: A Review of the Genus *Cancellus* (Crustacea: Diogenidae) with the Description of a New Species from the Caribbean Sea. By Barbara Shuler Mayo. Pp. iii+63. \$1.50. No. 151: Revision of Corophiidae and Related Families (Amphipoda). By J. Laurens Barnard. Pp. iv+27. 55 cents. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) [711]
 United States Department of the Interior: Geological Survey. Bulletin 1351: Geology and Description of the Thorium-Bearing Veins, Lemhi Oass Quadrangle, Idaho and Montana. By Mortimer H. Staatz. Pp. iv+94+plates 1-4. Professional Paper 716-E: Distribution, Thickness and Lithology of Paleocene Rocks in Pakistan. By Charles R. Meissner, Jr., and Habib-ur Rahman. Pp. v+64+plates 1-4. \$1.25. (Washington, DC: Government Printing Office, 1972 and 1973.) [711]
 The Coconut Industry Board, Jamaica, West Indies. 12th Report of the Research Department, July 1971-June 1972. Pp. 65. (Kingston: Coconut Industry Board, 1973.) J\$2; 90p; US\$2.20. [811]
 Bulletin of the American Museum of Natural History. Vol. 152, Article 2: Revision of Ground Beetles of American Genus *Cychrus* and Four Subgenera of Genus *Scaphinotus* (Coleoptera, Carabidae). By Tatiana Gidaszow. Pp. 51-102. (New York: American Museum of Natural History, 1973.) \$23.00. [1341]

nature

Volume 248

April 12, 1974

Science beyond the fringe

It would have amazed the Victorian steadfasts of science how confused some of our attitudes towards science still are. Instead of the logical world they hoped for and tried to work in there is a discernible tendency for the public and even some practitioners of science to turn their backs on science and become preoccupied with the bizarre and the magical.

Mr Uri Geller is only the most recent to cast doubt in the public mind on the efficacy of rational explanation.

Archaeology is being plagued by a series of ideas which have achieved a following particularly among the young. Professor Glyn Daniel has tilted against some of these nonsenses in his fascinating and always readable editorials in *Antiquity*. At one end of the spectrum there are innocuous extrapolations of conventional ideas about the significance of Stonehenge. At the other there are people busily poring over Ordnance Survey maps of Britain plotting mythical alignments between ancient monuments and erecting fanciful hypotheses about prehistoric technological civilisations. It is not as if archaeology needs enlivening—the radiocarbon dating method has already done that and caused great excitement by requiring revisions of old ideas about the relationships between prehistoric peoples.

Elsewhere, Velikovsky is enjoying a revival at a time when real astronomy and the earth sciences have never been more fascinating. How long will it be before psychiatrists are inundated with requests for more research into possession by devils from people who have seen the film *The Exorcist*?

It is difficult to know why these beliefs beyond science have such a following and whether it is genuinely on the increase, but presumably it is closely tied to a prevailing mood of the questioning of established values and a disbelief that science has more than marginal relevance to the human condition.

All this can be fun, of course, but the danger is that taken too seriously it detracts from all the exciting things that are being achieved in, for want of a better description, conventional science based on conventional ideas about the way a scientific investigation should proceed. And, when pseudo-scientific ideas are finally demolished, as they usually are (although one of their characteristics is that they are long in the dying) their demise casts doubt in the minds of the general public about the value of scientific research in general.

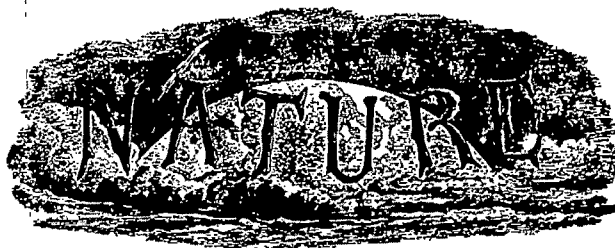
Scientists want to fight this distressing drift from the scientific way of thinking, but are not being noticeably successful. Part of the answer is to make real science more attractive, and for this reason scientists should not be dismayed when they find professional communicators of scientific ideas using imprecise words in place of the

precisely-defined terms from the science laboratory. To capture and convey the excitement of modern science is the main thing. In this respect Professor Kurti's proposal for a code of professional etiquette, published in the November 1973 issue of *Physics Bulletin*, may turn out to stifle scientists' communications with the general public and have the opposite effect to the one he desires.

Nature has a responsibility in all this; a responsibility that perhaps more than anything else guides us in the choice of papers for publication. If the successes of the scientific world are to come through clearly to the general public and appear more compelling than myth and idle speculation does, it is also vitally necessary that the community of professional scientists should understand each other. If scientists, fully aware of the scientific method, find the work of others in different disciplines boring and unconvincing there is little chance that the layman will feel otherwise. As far as we are concerned a scientific journal can help by encouraging its authors to explain their findings in a way that will make them as widely accessible as possible. After all, if a paper is not understandable outside the branch of science in which it originated the paper might just as well be circulated and filed as a private report. It is also a continuous surprise to us how coquettish some authors are when it comes to an assessment of the implications of their findings. Often papers are sent to us describing new measurements of the levels of pollutants and contaminants, but with no estimates of whether the estimated levels are dangerous.

If we are more persistent than most journals in importuning authors to be briefer or blunter this is partly the deeply-felt view that brevity and clarity ultimately lead to wider dissemination of ideas and a greater understanding of science and sympathy with its objectives.

100 years ago



On the Word "Axiom"

IN reference to the controversy between Mr. Spencer and his reviewer about Sir I. Newton's calling his laws of motion "axioms," it is to be observed that there is a certain ambiguity in the word. "Axiom" is from ἀξίωμα (I demand), and would thus signify a first principle to be taken for granted. It does not, of course, carry with it the meaning of a necessary judgment which cannot be contradicted. Whatever may be considered the ground of Euclid's "axioms" so called, Euclid himself did not apply that name to them; but the first nine he called "common notions," and the last three (which are peculiar to geometry) he placed among the postulates (ἀποδείγματα), and heads them with "let it be granted." Now it is clear, from Newton's own words, that in calling his *Leges motus* "axioms," he does not imply that they are necessary judgments, but that he requires them first of all to be granted (however established) in order to the following reasoning. In other words, they are postulates, like Euclid's last three "axioms." In our modern use of the words "axiom," "axiomatic," there is always implied the ground why a proposition is demanded as granted, viz., because its necessity is self evident; but this wider use is not required by etymology, or (I think) in interpreting all ancient writings. F.M.S.

From *Nature*, 9, 462, April 16, 1874.

The problem of Soviet scientists: a reply to John Ziman

Professor Eric Burhop, of University College, London, replies in an open letter to points raised in an earlier article on relationships with Soviet scientists.

DEAR JOHN,

Although there may be many things wrong with the organisation of science and the attitude toward some scientists in the Soviet Union, I do not feel they warrant the kind of special action you propose in your article in *Nature* (246, 322; 1973).

In the first place your article is tendentious and contains a number of inferences which I believe you would have difficulty in establishing. It would indeed be a matter of concern if Soviet science "were to fall into decay and disrepute". Of course if, like Solzhenitsyn, we were to hark back a quarter of a century, there might be some cause for concern. But the Soviet scientific community has itself been able to correct the most glaring of the aberrations that gave rise to anxiety at that time. The difficulties it faces today, while no doubt often stupidly frustrating, are not of the kind likely to threaten Soviet science with "decay and disrepute". The reasons Soviet scientists often turn up late at conferences or do not turn up at all is mostly, though admittedly not entirely, due to a clumsy bureaucracy which affects not only scientists. These are the kinds of things—basically trivia, however maddening they may be—that can only be effectively dealt with by the Soviet scientists themselves. I agree that it would be beneficial if a greater number of Soviet scientists were able to come to conferences abroad. I think the position is improving but the problem is very largely one of foreign exchange of which there is a great shortage, affecting all travel. It would certainly be a more sensible use of the available foreign exchange to allow a larger amount for travel of scientists but this is not the kind of thing that can properly form the basis of anything approaching a demarche by the Royal Society.

Some criticisms you make are serious ones with which I would not disagree, but the situation is more complex than you imply. You ignore completely the differences in values characteristic of Western and Soviet society. For example, in Western society the increase of private wealth by speculation is regarded as normal, or even laudable. In Soviet society it is punishable very severely. In Western society too much concern for problems of peaceful coexistence, disarmament and the abolition of nuclear weapons is regarded as at best naïve and at worst subversive. In Soviet society these things are highly regarded.

In a society based on social ownership of the means of production, the operation of a planned economy is likely to require more restrictions in some directions than in a society based on the vagaries of the free market. Many people, including I believe many scientists or writers, may feel this a price well worth paying for a society founded on socialist principles, in which continued social, economic and cultural advance is assured. They may indeed assess these as far less onerous than the 'restrictions' inherent in inflation, unemployment and social insecurity which are unknown in their society but all too real in our own society.

Many of the disabilities to which you refer are related to the question of the emigration of scientists to Israel. The Soviet Union has consistently supported the Arab countries against Israel and this policy seems to have very wide support

among Soviet people who tend to regard those Soviet scientists who wish to emigrate at the present time and are presumably willing to help Israel in the war in somewhat the same light as British people on the whole regarded Oswald Mosley in 1939. It may be regrettable, but hardly surprising, that their colleagues have in some cases refused to work with scientists who have applied to emigrate to Israel. This is surely a passing phase and if and when a stable peace comes to the Middle East the campaign against the emigration of scientists to Israel will abate.

Arguing that the Council of the Royal Society should intervene, you say "such a statement would make it quite clear to the main body of Russian scientists that we understand their present position . . .", implying that they would welcome such a statement. I do not believe there is a basis for such a conclusion. I have come into contact with many Soviet scientists and I know that the vast majority would not welcome such intervention. Indeed this has been pointed out to me quite passionately by some eminent Soviet scientists who are by no means complacent about some of the aspects of the situation to which you refer. They feel that an intervention of the type you envisage would certainly make things worse by strengthening more 'hard line' as opposed to more 'liberal' tendencies.

The most important reason for opposing intervention is, however, surely the effect it could have on the development of scientific exchanges. You place very little store on these exchanges. Others, however, have had a different experience of them. The interests of both British and Soviet science require an extension, not a cutting off of such exchanges. There is, however, an even more important reason for fostering international scientific cooperation in general and cooperation between the Royal Society and the Soviet Academy of Sciences in particular. They are an important factor in building relations of friendship and understanding between our two peoples. They can assist in breaking down the fortress mentality which largely colours Soviet attitudes to the outside world. This attitude has been built up over the years as an understandable consequence of political attitudes towards the Soviet Union. It constitutes a major obstacle to the development of the easy, informal relations between Soviet colleagues and their opposite numbers in the West. I know you would like to see these relations develop and yet I fear that the kind of campaign you are sponsoring will have the effect of strengthening rather than loosening this fortress mentality.

For the Royal Society to intervene would represent a departure from long established policy. All through the Vietnam War it said not a word about the way in which modern scientific techniques were applied by the United States Army, assisted unfortunately, by many United States scientists who should have known better, in the development of defoliants, virus diseases of growing rice crops, riot control weapons, the electronic battlefield. In Chile at the present time the military junta has taken over the universities and many university staffs, including scientists, have lost their jobs, have been forced to emigrate, or even put in danger of their lives. Recent press reports make it clear that the United States Government continues its programme of 'behaviour modification' experiments on prisoners—a gross professional abuse of psychiatry, as you rightly say. I have not heard suggestions that the Royal Society should say anything about any of these things.

In my opinion, before the Council of the Royal Society starts making public statements about the matters you raise in your article it might appropriately look at things in Britain. I do not wish to exaggerate but, nevertheless, there are blemishes sufficiently serious for the National Council for Civil Liberties to feel impelled to set up a Council for Academic Freedom and Democracy here.

Yours sincerely,

ERIC BURHOP

international news

A CONTROVERSIAL scheme to explode a string of nuclear devices underneath the Colorado Rockies to extract vast deposits of natural gas has run into a critical snag. A key test of the technique, a 90-kiloton blast called Rio Blanco which was set off last year amid a storm of protest, has turned out to be something of a flop, and officials of the Atomic Energy Commission (AEC) are now trying to find out what went wrong.

The gas is trapped in small pockets in a layer of sandstone about a mile underground, and there is reckoned to be enough under the Rocky Mountains to supply the entire demand in the United States for about 10 years. But the sandstone is not porous enough to allow the gas to be extracted simply by drilling into it, and so the AEC together with an industrial partner, has been trying to free the trapped gas by smashing the rock with nuclear explosives.

The idea is to fire off a nuclear device in the gas-bearing sandstone, which creates a large underground cavern and fractures the surrounding rock for hundreds of feet in all directions. Gas then seeps into the cavern through the fractures and it is later pumped to the surface.

Rio Blanco was the third test of the technique, but it was the first to employ a radically new idea to improve the gas yield. Instead of firing off a single nuclear device, three explosives were placed in a vertical line, about 450 feet apart, and triggered simultaneously. The hope was that the caverns produced by each explosion would link up to form a huge cylindrical chimney in the sandstone layer, about 150 feet in diameter

Rio Blanco draws a blank

Colin Norman, Washington

and 1300 feet deep, surrounded by massive fractures radiating outwards.

But earlier this year, when the AEC began to extract gas from the chimney, it found that it was getting a yield only from the cavern created by the topmost explosion—somehow, the caverns either failed to link up or they had later become sealed off from each other.

Asked last week what went wrong, J. Keith Davey, an AEC official concerned with the project, said that several things could have happened. The simplest explanation is that the explosives were placed too far apart so that the caverns they created just didn't join up, but another possibility is that the chimney could have become blocked.

It is possible that the glassy melt formed by the intense heat of the nuclear explosions didn't fall to the bottom of the chimney, but blocked off the connection between the caverns. Another likelihood is that the rocks simply shifted after the explosions as Davey points out, underground mechanics at that depth are not well understood. Still another possibility is that the chimney could have been disturbed when the borehole was sunk into it to pump the gas to the surface, a suggestion which is given some credence by

the fact that about 12,000 barrels of lubricating mud were lost during the reentry operation.

Later this year an attempt may be made to sink an oblique shaft into the second cavern to try to provide some answers, but whatever went wrong the failure has greatly damaged the chances that nuclear gas stimulation—as the technique is called—will ever be turned into a commercial venture. The scheme has already run headlong in spirited opposition from several diverse groups, and technical problems at this stage could well send it to an early grave.

Rio Blanco itself was entirely an experimental test, but what is worrying opponents of nuclear stimulation is the prospect of a full-fledged commercial venture. To exploit the gas field under the Colorado Rockies entirely would require several thousand nuclear blasts, a prospect which pleases neither the people living in the area nor the oil companies who are hoping to produce oil from the shale fields which happen to sit immediately above the gas-bearing sandstone.

A repeat of the Rio Blanco test would be sure to attract a huge amount of opposition, particularly in view of the fact that two oil companies have just paid nearly \$210 million for the right to set up a prototype oil shale process on a small strip of federal land not far from the Rio Blanco test site. Alternatively, the AEC would be hard put to justify moving to a pilot scale nuclear stimulation operation on the basis of the Rio Blanco results. The failure is thus a critical blow to the whole enterprise.

Cash on the rail

Rail from Ascension Island, drawn by the explorer, Peter Mundy, 1656



WHEN does specimen collection of rare birds and animals for scientific study become unacceptable? This question has been exercising ornithologists on both sides of the Atlantic recently. The trouble seems to arise when enthusiastic ornithologists and museum staff visit remote places and offer cash and other inducements to the natives for rare birds dead or alive.

The most recently reported case concerned a member of the American National Museum of Natural History of the Smithsonian Institution, who, during a visit to St Helena for the admirable purpose of studying the fossil remains of flightless rails, admitted offering cash rewards after the natives re-

ported the presence of a living bird of this type. No specimen was in fact forthcoming and the author says that he is convinced that the bird was imaginary. But he might remember Charles Kingsley: "no one has the right to say that no water babies exist, till they have seen no water babies existing; which is quite a different thing, mind, from not seeing water babies".

Elsewhere in his article, the author says that the flightless birds of these remote islands were certainly only made extinct after men arrived. The Smithsonian seems to be following the Victorian idea that an animal is not discovered until it is safely stuffed in a museum.

Around the Solar system in 1,800 days

FOLLOWING the complete success of Pioneer 10, NASA has decided to re-target Pioneer 11, now en route for Jupiter, rather than simply to duplicate the earlier mission. As a result, Pioneer 11 will now swing by Jupiter, using that planet's gravity to assist it into a new orbit and a rendezvous with Saturn late in 1979. And that will pave the way for the planned Mariner missions to Jupiter and Saturn, which include the possibility of putting at least one of the Mariner craft in orbit about Saturn.

Pioneer 11 has now passed the asteroid belt and is more than 4.5 astronomical units (AU) from the Earth; its new path should take it to an encounter with Jupiter on December 2 or 3 this year. The latest Pioneer will pass much closer to the cloud tops of Jupiter than its predecessor (27,000 miles as against 81,000 miles, according to a report in *Aviation Week & Space Technology*, 100, 18; March 25, 1974). This close approach is essential if good use is to be made of the slingshot effect of Jupiter's gravity and using such approach can only be contemplated in the light of the Pioneer 10 data. Those data showed the structure of magnetosphere and radiation belts sufficiently clearly that the Pioneer project scientists are confident that Pioneer 11's new path will take it through a gap in those belts, so that in spite of its close approach to Jupiter it will receive less damaging radiation (by some 75%) than did Pioneer 10.

But the actual path followed by Pioneer 11 after the Jupiter encounter is almost as interesting as the objective itself. Like the recent Mariner 10 Venus/Mercury mission, which was the first to utilise a gravity-assisted trajectory, Pioneer 11 will actually be slowed down by its encounter with the first planet it passes (Jupiter). The new path will take the Pioneer across the leading edge of the planet (Pioneer 10 passed the trailing edge and was accelerated above escape velocity for the Solar System) and in a loop back

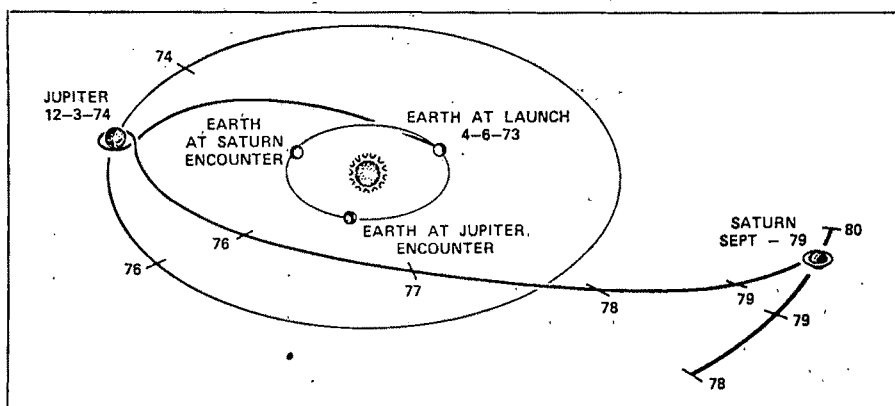
across the Solar System, to meet Saturn, eventually, on the opposite side of the Sun from the Jupiter encounter.

The slow journey back across the Solar System will take nearly five years, with Saturn being reached some time in October 1979. The closest approach to the Sun will be some 3.5 AU but the space probe will not have to face the hazard of the asteroid belt again, since its new path will take it out of the ecliptic plane. Clearly, this will provide a wealth of information about interplanetary space. But what are the prospects for the Saturn encounter itself?

Saturn orbits some 9.5 AU from the Sun, at which distance radio signals take 1.5 h to reach Earth. It would be asking too much to expect spectacular information to be relayed by the first probe to visit Saturn—but as long as the radio transmitter is still functioning useful data could be obtained simply by flying Pioneer 11 through the rings of Saturn to see if it hits anything. That would at least indicate the limits of safe orbits for the following Mariner craft.

It looks as if that at least should be possible, and some scientific information may also be gleaned as a bonus. The spacecraft's nuclear isotope generators were designed to produce 100 W five years after launch (April 1978 in the case of Pioneer 11) but judging from the experience gained by Pioneer 10 the generators are likely to last rather longer than anticipated. It seems feasible that all the experiments could still be operating in October 1979, with sufficient power available to relay their findings; and perhaps even pictures, over Pioneer 11's 10-W transmitter.

FOOTNOTE: It seems that the *Sunday Mirror* has even more faith in the versatility of NASA spacecraft and the power of the gravity-assist technique than NASA project scientists themselves. In the March 31 edition of that newspaper we were presented with a Mariner 10 picture of Mercury with a caption which ended "Mariner 10 is now hurtling through space ready to take pictures of Saturn, the ringed planet . . . in 1979".



Mars Sampler in 1981?

NASA engineers and scientists will be meeting in Washington on May 23 and 24 to discuss the feasibility of a unmanned Mars sampling mission. Such a mission could be flown as early as 1981 if there is cooperation between Soviet and American space agencies.

The prospect of complete collaboration between the two giants has been growing for some time, and the Mars project seems a logical next step. The mission divides clearly into two parts; one country could take responsibility for the launch from Earth and delivery to Mars of the sampler package while the other looked after that package and produced the means of getting the sample home.

Some of the possibilities which will be trashed out in detail in May are mentioned in *Aviation Week & Space Technology* (100, 14, April 1, 1974); support for the proposal is likely to be strong both because of the desire to develop from the 1975 Viking lander (assuming that is successful) and because the returned package need only be placed in Earth orbit, where it could be collected by the shuttle. But options are being kept open on such projects as Viking 1979 (*Nature*, 248, 15; 1974), and it remains to be seen whether the Russians would accept the proposal of a joint mission to bring back Mars samples.

Ring of little confidence

John Gribbin

SHOULD the Science Research Council (SRC) support the building of an electron storage ring at Daresbury to provide a central synchrotron facility when the electron accelerator NINA closes down in 1978? Taking advantage of a spare afternoon at the end of a conference on synchrotron radiation held at the University of Reading under the auspices of the Institute of Physics, the SRC case study and plans for such a facility were put to the physics community on April 2. Because of a clash of dates with a Chemical Society meeting, the proposal has still to be discussed with rank and file chemists. But the Reading meeting showed clearly that at the very least the proposal needs careful rethinking and emphasised the value of such discussions with the people who would be using such machines.

As at present conceived, the new machine would cost some £2 million to

build, with running costs in the vicinity of £750,000 a year. For this money, the facility would provide the first such source in Britain dedicated to synchrotron work—present synchrotron users at Daresbury and elsewhere are very much aware of their status as 'parasites' on the high energy machines.

Ten beam lines, each capable of supporting two projects, would eventually be available but it would take some 10 years to work up to this fully operational capacity. The machine would operate at 2 GeV with an electron current of 1 A, the planned radius of the electron orbit being 15 m, the bending radius of the magnets 5.55 m, the magnetic flux 1.2 T (12,000 gauss) and the total radiated power 240 kW. All that is impressive enough, as is the list of possible experiments which could be carried out with such a facility. But is it the best way to spend the available money?

Many of the physicists present at Reading expressed doubts about this. The most crucial question, which is not yet answerable, is just when the facility could begin operations. Both for economic reasons and because of the community which has built up around Daresbury, the plan envisages that the new ring would be built inside the NINA hall. Inevitably, that means that there must be a delay between NINA closing down and the new machine becoming operational. The optimists suggested, in the SRC plan, that the delay might be a year or so, provided work begins on the project soon. But few, if any, of the synchrotron users believed this. B. Bleaney (University of Oxford) went so far as to say that he would bet on a gap of more like four or five years in which British synchrotron workers would have no major facility in this country; there were no takers prepared to accept his bet.

That raises the question of just how useful such a machine will be in the mid-1980s. Certainly everyone present at Reading would have been delighted to have such a facility now but fears were expressed that European and American machines now being planned and built might have tackled the most exciting problems before the new British machine got a look in. Of course, such machines will be extremely useful for the foreseeable future, just as conventional X-ray sources, for example, have not been rendered obsolete by synchrotron facilities. But it is one thing to build such a costly installation, do the exciting work and then continue using it into a productive middle age, and quite a different kettle of fish to build an expensive facility knowing that it will probably only ever be used for routine, if valuable, work.

There was also some confusion about just how much the costs would be, in terms of experiments using the facility.

It was put to the SRC representatives that although their plan did not spell out the financing in those terms, a generous interpretation of the running costs, together with the proposal that ten "new projects" might start up each year, meant that each project had to be "worth" £100,000. That is a lot of project—something like three or four times the cost of typical projects being run in universities in the areas of research expected to benefit from the new facility. Even allowing for Parkinson's laws, is it really sensible to plan for such growth?

But there are two points to be considered against this apparently large expenditure. First, if only 100 people are using the machine, then its running cost of £750,000 a year breaks down as £7,500 a person a year—more the sort of figure which the average person can understand and, it seems, not a sum to make the SRC blanch. Second, at present, experiments funded by the SRC in universities have invisible means of support—they do not have to pay for buildings, for electricity, for non-scientific staff to keep the buildings running and so on ("heat, light and sound", as a wag in the audience put it). And in the present economic climate it is best, perhaps for the SRC to be seen to stand on its own two feet.

This problem, although slightly out of the mainstream of the Reading meet-

ing, is an interesting and tricky one. For, if the SRC grasps the nettle firmly and says publicly that its expenditure plans are based on the assumption of less university support, such bodies as the University Grants Committee will surely take note and reduce their allocations accordingly. On the other hand, if the SRC assumes continued support of this kind at present levels then it will be severely embarrassed if such support is not forthcoming.

It is certainly good to see the SRC encouraging open discussion at the early stages of planning such projects as the new storage ring and it was clear that both the SRC and the physicists were able to learn about each others' difficulties and needs at Reading. The plan as originally envisaged seems unlikely to survive and a powerful case can be made for pressing ahead more vigorously with such a project, perhaps at a different site, if there is to be such a ring at all. It also remains to be seen how the need for such a large sum might inhibit other activities by the SRC's Physics Committee—it is not difficult to think of ways of spending £2 million which would be at least as useful as building a synchrotron facility. But certainly there is less chance of ending up with an underused white elephant when the committee is taking such a receptive attitude to frank comments from the physics community.

Business report *Roger Woodham*

A HEALTHY increase in capital has been announced by Imperial Chemical Industries (ICI), following the publication of its annual report for 1973. The amount sanctioned is £250 million, 60% of it earmarked for the United Kingdom where the major project started in 1974 may well be a giant ethylene plant on Teesside producing 500,000 tons a year. This was originally to have been a three-way affair—Shell, ICI and BP—but Shell has now withdrawn for undisclosed reasons and a firm agreement has not yet been made between ICI and BP. Will intercompany squabbles spoil the attempts to avoid a recurrence of the plant overcapacity problems of recent years?

If all goes according to plan ICI will be livening up its investment in spite of Mr Healey's Budget. Just how things have ground almost to a halt recently is highlighted by figures in the 1973 report for fixed assets—plant, equipment, buildings and so on, the stuff of investment. In 1973 fixed assets increased from £1,160 million to just £1,185 million. By contrast, in 1964, 1965 and 1966 the assets in use were increasing by leaps and bounds, from £646 million to £760 million to £880 million.

The report also reveals some interest-

ing trends in ICI's sales, worth £2,166 million last year. Whereas in 1963 the United Kingdom accounted for 52% of the sales, in 1973 the proportion was down to 43%. Allowing for imports of raw materials and so on, ICI contributed positively to the British balance of payments by some £220 million.

●HELICOPTERS landing on oil rigs, and possibly ships entering estuary ports, may benefit in a few years from the improvements that continue to be made at the Mullard Research Laboratories, Redhill to its Microwave Aircraft Digital Guidance Equipment (MADGE). The basic system has NATO's blessing and is being manufactured commercially.

A substantial proportion of one of MRL's four divisions, which between them spend £3 million a year, is engaged on work of broadly this kind, sophisticated radar. The beauty of MADGE is that the stationary part of the system, which is 'interrogated' by a microwave signal from an aircraft (or ship) can be set up in only 15 minutes or so. Ideal, as NATO evidently thinks, for landing and takeoff near the front line, but also a good bet for civil aviation.

LATE LAST year, when Arab countries suddenly shut off oil supplies to the United States, the Nixon Administration and the United States Congress reacted predictably by espousing a pell-mell drive to make the United States independent of foreign suppliers of energy so that the country would not get caught short again. President Nixon dubbed the effort "Project Independence", and optimistically said that the goal of self-sufficiency could be reached by 1980—a prediction which he continues to make even though his own officials have publicly stated that it is totally unrealistic.

A striking aspect of the debate about Project Independence is that it has centred almost entirely on the levels of funding that have been proposed and on the way the project should be managed, but few people have publicly challenged the philosophy behind the enterprise. Like God, motherhood and apple pie, energy self-sufficiency is tacitly assumed to be good for America. But a report published last week by the Ford Foundation may change all that.

A preliminary publication designed to stimulate debate about the energy policy choices that now confront the United States government, the report says that the decision "to rapidly develop all federal energy resources simultaneously . . . may make sense in some areas, but as an across-the-board proposition it represents a failure to weigh conflicting values". For one thing, it may result in unacceptable environmental degradation, and for another, it may have damaging repercussions on United States foreign policy. Moreover, the report points out that Middle East oil can be produced at very low cost, and although at present prices it may seem economic to extract oil from shale and coal, there is no guarantee that these products would not be undersold by Arab oil in an open market.

Prepared by the Ford Foundation's Energy Policy Project—a \$4 million study which should be completed later this year—the report is an attempt to suggest that the United States is not "bound in an energy straitjacket" but has a number of policy choices available, ranging from a gradual progression to zero energy growth on the one hand, to aggressive development of new energy resources, so that Americans can continue to increase their consumption of energy virtually unchecked, on the other, the report outlines some of the social consequences of the options.

If the United States continues to increase its energy consumption at the same rate as in the golden years preceding the crisis—about 3.4% a year—production will have to be doubled by the year 2000. According to results of a study carried out by Resources for the Future, Inc., such a target could be met by an aggressive programme of

developing domestic sources of energy, including oil from the outer continental shelf, nuclear technology and shale oil. There is, however, some question about the availability of fossil fuels much beyond the year 2000 if they are exploited at maximum capacity for the next 30 years, and the energy demand could only be met by abandoning some environmental standards.

At the other extreme, the United States could move gradually towards

Ford spells out the energy choices

Colin Norman, Washington

zero energy growth by developing more efficient ways of using energy and by cutting back on consumption. If such a path is followed, it would mean some rather fundamental changes in the American way of life, but the report suggests that the goal could be met without dire consequences, either to individuals directly or to the economy of the United States. In fact, the report states that "because of its greater efficiency in using energy, the [zero energy growth] scenario would include many more energy benefits than the nation now enjoys".

A key to this optimistic prediction is an orderly progression to zero growth, rather than a sudden dislocation, so that the goal will be reached towards the late 1980s. By that time, individual energy consumption would have risen by about 10% from the present level. "Managing a transition to zero energy growth appears to be possible if the change takes place gradually over ten to twenty years as part of long term planning and a growing social consensus as to its desirability", the report states.

To achieve the goal of zero growth changes must be made—for example people would have to spend less time travelling, efficient rapid rail links would have to be developed between cities, there would have to be a trend toward multi-family housing, the growth of energy-intensive industries would have to be slowed down, there would have to be more recycling of valuable products such as aluminium and the use of plastics would have to be curtailed. Clearly, such changes will not come easily and will demand a fundamental change in governmental thinking to ensure that an orderly transition is made to "a society that husbands its resources, including energy".

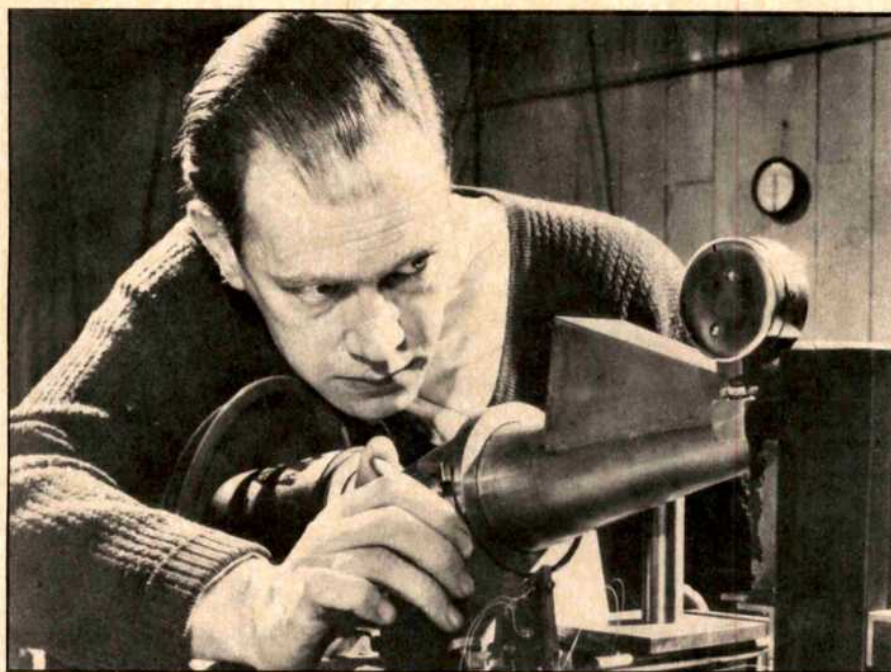
Between those two extremes lies the possibility for increasing energy production while reducing the growth in demand. This scenario is called the "technological fix scenario" in the report and in a free enterprise economy it is probably the most likely course for the country to follow. To illustrate the choices available, if such a path is followed, the report outlines some of the decisions which will have to be made to cut the rate of growth of energy consumption to half that pertaining before the energy crisis began to bite.

One fundamental fact to emerge is that although some research and development will be required to develop technologies for conserving energy—such as solar heating and cooling, and heat pumps for residential heating—"the major uncertainties are political, institutional and economic questions rather than physical or technical limitations". Which is a little like saying that the United States could move from a capitalist to a socialist economy but for political constraints.

Another important aspect of the technological fix scenario is that with an aggressive programme of energy conservation, choices can be made between competing technologies for energy supply. For example, the report points out that if strip mining of coal in the western United States is considered to be too destructive to the environment, other sources of energy could be expanded to fill the gap.

Alternatively, the report mentions in several places that fundamental questions have arisen in the United States about the safety and environmental risks associated with nuclear power, and it suggests that a moratorium could be called on the building of new power plants until such issues are resolved. Since the United States Atomic Energy Commission (AEC) is basing its case for the fast breeder reactor chiefly on the fact that it will supply a huge proportion of the country's energy requirements by the end of the century, the AEC will not take very kindly to the suggestion that the entire nuclear programme could be abandoned without plunging the United States into total darkness.

The fact that such options exist has largely been closed over in the debate about Project Independence, and the chief result of the report will be to strengthen the hand of environmental groups in their opposition to some of the more damaging energy projects. But this document is simply a discussion device, and the facts behind the analysis will not be published until late this year. In particular, the final report will present a more closely argued analysis of the consequences of a transition to zero energy growth.



Professor R. V. Jones. A wartime picture by Larry Burrows

Concurrence in learning and arms

David Davies

It was the reunion of the Class of '45 at a recent meeting of the Royal Society to discuss the effects of two world wars on the organisation and development of British science. No gathering of reminiscing fighter pilots could match this occasion, where the boffins, with their H2s, operations research, radar and the Manhattan project, assembled once more to draw the morals, not in a smoky bar room but in the clinical jumbo jet interior of Carlton House Terrace. Two Lords, two Members of the Order of Merit, seven knights and all Fellows of the Royal Society, except the educator, the historian and the industrialist. The high-spot seems to have been the night before when the speakers gathered round the dinner table to swap stories—not, one of the participants pointedly told me, the sort of stories to be repeated within earshot of the Editor of *Nature*. Nevertheless there was sufficient vigour left on the day to delight an audience of two hundred.

Before the twentieth century war had practically no impact on science, nor science on war. R. V. Jones (who had convened the meeting) named the few exceptions—Bacon had advocated "concurrence in learning and arms", Babage and Playfair, the extraordinary precursors of so much, had concerned themselves with science in warfare and Kelvin and Rayleigh advised the services. Organised science was still small; the Cavendish was getting by on £1,500 a year just before the First World

War (WWI), including demonstrators' salaries. There were terrible shocks to British complacency in WWI—gas, Zeppelins and U-boats were three scientific and technological tools of war which had to be countered. Science was also badly hit by the loss of Moseley and Hopkinson. Scientists were wasting their talents as cannon fodder and in the Second World War (WWII) a register of scientists was maintained.

The successes of WWII, particularly radar, finally ensured that science was moved closer to policy making. Lindemann was the first scientist since Playfair to be involved at governmental level and the need to share scientific results led to the dispatch of A. V. Hill, Ashby and Needham to Washington, Moscow and Chungking.

It is easy to see the importance of WWII because many of the participants are still around but WWI had an equally profound effect on science, as D. S. L. Cardwell pointed out. Germany had encouraged science-based industry and Britain's dependence on Germany in such matters as dyes, pharmaceuticals, instruments, fine chemicals and so on led to grave shortages. New ventures were launched—British Dyestuffs, research associations for industry and the Department of Scientific and Industrial Research (DSIR)—and by the end of the war the importance of science in education was realised. British science got a leg up from WWI but science in general lost much of its supranational character.

What sort of leg up did science get from WWII? This question occupied speakers for the rest of the day. The Second World War, it seems, gave a boost to scientists rather than science. For science itself there were obvious

gains such as nuclear physics, but also less obvious losses (C. H. Waddington held that without WWII molecular biology would be fifteen years older, Bernal and Astbury, not Watson and Crick being the midwives). In technology too there were both gains and losses, as D. S. Davies (ICI) detailed in a thoughtful paper. Prewar inventions such as titanium technology, jet engines and radar were clearly advanced but against this were setbacks in television, synthetic fibres and plastics. A static gross domestic product inhibited investment in special plant in the war, of course. Of specifically wartime inventions, maybe operational research was the only one which has so far had a major impact on Britain.

The effect on the scientists had, however, been colossal, both in the way they went about their science and in the way they ensured that government took note of them. J. A. Ratcliffe and Sir Edward Bullard told broadly similar stories. The scientist who in 1939 left a laboratory used to making do with no phone and no secretary, used to the idea that science was gentlemanly, cheap and simple and used to being rebuked by the chief assistant for using expensive wood to make breadboards ("mahogany, Mr Ratcliffe?") came back a changed man. He had learnt how to get his way, how to coordinate a team and how to use communications. The outward sign of this was the growth in numbers of research workers and the even more spectacular growth of the cost of research projects. As for biology, even though, as J. W. S. Pringle put it, this was not a biologist's war it changed the thinking of many who found pure research not so important and the possibility of doing new things and playing a role in public affairs attractive.

It was inevitable that American science should have come in for mention. Before WWII, said Lord Bowden, America had been regarded as subordinate to Europe in science but the vision of men such as Vannevar Bush and a willingness to buy scientists, if necessary, swung the pendulum. Besides, nuclear energy, the first big science, was growing and, as Sir Harrie Massey described, the Manhattan project sowed the seeds for a whole new approach to scientific operations in which the United States had a clear start. The Second World War had given many scientists a sense that what was possible in principle was also possible in practice. In wartime, of course, this did not have to be profit making; the carrying over of this attitude into peacetime has not necessarily led to the science-based industries hoped for.

R. V. Jones considered government establishments in a second paper. The aim before WWII had been to keep

government and university scientists close together, but after WWII, despite early hopes that teaching and fundamental research could have priority over defence research, the government gained at the expense of the universities.

In 1931 there were 1,053 civil service scientists. By 1961 there were 15,474. Jones was critical of the trend and contrasted it with the United States, where nongovernmental laboratories had been fostered in the 1940s. Overenthusiastic claims for the Comet plane, thermonuclear fusion, the Einstein redshift in the Mössbauer effect and carbon fibres had not helped the reputation of the

establishments. In wartime they flourished because of the spirit of collaboration. Not so in peacetime when the spirit was competitive.

Finally, how did scientific advice to the government change as a result of the war? Lord (*alias* Solly) Zuckerman sketched the transition from the advice, often informal, of the thirties, of Lindemann, Tizard, Blackett, Bernal, and so on, through the war when many scientists became planners with operational research and a few were involved in the determination of national policy, to the post war period with the greatly enhanced reputation of the scientist in government. Science advisers were

brought in all over the place but their utility was restricted as they were rarely allowed to be more than technical assistants, kept out of the bigger issues of government. This trend culminated in the creation of a Chief Scientific Adviser in 1964, though if there were an issue on which he and trade union leaders had something to say, the trade unionists could be expected to carry more weight.

A fascinating meeting. Too bad the youngsters chose to stay in their laboratories. Those with scant regard for the history of science as a proper study (and there are many) would have been won over by this delightful occasion.

correspondence

Nuclear simplicity

SIR—The paramount command for nuclear power reactors is safety. The next most important requisite is the lowest possible cost of the electric power actually supplied to the grid.

Safety depends directly on simplicity and so does the cost of safety. An inherently simple plant can be made safe more easily and at a lesser cost. Simplicity also favours high availability and thus a lower unit cost of electrical power.

Simplicity is rare because new ideas are rare. The original safety containment concept was based on countering the hazard of brute force by brute strength until, quite late on, somebody hit on the idea of providing a condensational pool. The same is true of the coolant recirculation pumps: first external pumps with large connecting pipes, then external pumps with internal jets and small pipes, then axial internal pumps without any pipes. Pipes may fracture. In both cases the simpler and, with hindsight, very obvious concept resulted in lower costs and much greater safety.

I owe the concept of ponnery to my children who used to build edifices of chairs, saucepans, the cat's basket, vacuum cleaner and so on, up to the ceiling, one 'pon the other. Such an edifice is called a ponnery. I now classify nuclear reactors into 'naturals' and ponneries.

The naturals are simple and obvious. I know of only two: the boiling water reactor (BWR) and the gas-cooled fast breeder (GFB). The pressurised water reactor (PWR), the heavy water reactor (HWR) and the sodium-cooled fast breeder (SFB) are ponneries.

The BWR works on a direct cycle.

Disadvantage: active live steam. The main activity (^{16}N , generated by activation of oxygen) is, however, so short-lived that only the head end of the turbine need be shielded. Advantages: inherent, very rapid reactivity control (voids ratio), so that load follow-up is automatic and power excursions are self-limiting. No primary/secondary heat exchangers, so, no unnecessary thermodynamic losses and no leaky tubes, therefore high availability.

The PWR is, by comparison, a ponnery. It has a complex primary cycle, huge primary/secondary heat exchangers (very expensive and very leak-prone) and a low thermodynamic efficiency factor. High primary pressure (expensive), very modest live steam pressure (inefficient). From the nuclear point of view, sluggish reactivity control, hence the need for boron poisoning, hence a further sprouting of the ponnery to take the boron out again. It is inherent in a ponnery that the more you try to improve it the more profusely it sprouts.

The HWR introduces yet a third compartment, leaks from which must be controlled particularly carefully because heavy water is expensive. This is a ponnery within a ponnery. Its only advantage is the use of natural uranium. Once European enrichment facilities become available, this issue will be dead.

The obvious successor to the BWR is the fast breeder. But which one? The SFB is an obvious loser. It is of necessity a three-cycle system. Sodium is not a natural answer for transferring heat to water.

The natural winner seems to be the GFB, especially at high temperatures (process heat) and in combination with helium turbines (direct cycle, high effi-

ciency, minimum waste heat dumping problems). The technological problems associated with high temperatures (950°C and more) are worth solving, in contrast to those of the SFB. In its prime, perhaps 10 years from now, the direct cycle GFB should prove even simpler than the BWR.

Yours faithfully,

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Fishy correlations

SIR,—In reference to the article *What's in a Name?* (*Nature*, 246, 385; 1973) and G. Curzon's letter (*Nature*, 247, 82; 1974) citing contributions to this field by brain research workers, the pioneering work of American ichthyologists must not go unmentioned¹⁻⁴. Indeed the more than 300 ichthyological publications of Theodore N. Gill (1837-1914) make him perhaps the most productive investigator in this esoteric science.

Yours faithfully,

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¹Fish, F. F., *Furunculosis in wild trout*; *Copeia*, 1937(1), 37 (1937).

²Fisher, G. C., *Salt-water minnow in fresh water*; *Copeia*, 1920(79), 18 (1920).

³*A complete treatise on artificial fish-breeding* . . . (edit. by Fry, W. H.), (New York, 1854).

⁴Pike, N., *Notes on fishes of the ocean*; *Trans. R. Soc. Mauritius*, n. s. 7, 27 (1873).

news and views

When did plate tectonic processes begin?

A POINT often underemphasised by earth scientists, presumably because they think it too obvious to mention, is that their view of the Earth's behaviour is still heavily biased by data relating to the comparatively recent past. That the bias exists is, always has been, and probably always will be, inevitable, if only because younger rocks are generally the more abundant, the less altered from their original states and the more easily associated with processes whose occurrence is beyond doubt. But there are dangers in it nevertheless. For one thing, the more biased the results towards a relatively restricted range of time, the greater is the inclination to succumb to an uncritical belief in some uniformitarian world view and all that implies in terms of extrapolating the present into the past. It is perhaps significant that as geologists have become more familiar with the pre-Mesozoic and even the pre-Palaeozoic they have become less concerned to defend uniformitarianism or actualism (between which nine out of ten earth scientists cannot tell the difference anyway) and more confused about what these terms really mean or ever meant (except in terms of a reaction against biblical catastrophism). As a result, 'uniformitarianism' has now come to represent little more than a vague attitude, a negative thought that whatever uniformitarianism is it is not catastrophism. On the other hand, so strong is the historical influence that what may seem to be no more than an outmoded philosophical idea may still have deep-rooted behavioural repercussions.

The attempt to extend plate tectonic concepts further and further into the past is a good case in point. The widely-accepted group of ideas known collectively as the new global tectonics is based on data which relate to only the past few hundred million years, or less than 5% of the Earth's history; yet these concepts have succeeded in converting most earth scientists from a belief in a static Earth to a conviction that the planet is on a state of more or less continuous mobility. But however impressive and conclusive the evidence, such a radical transformation is the world view based on such a small proportion of the Earth's existence inevitably raises the question of what is the Earth's 'normal' long-term behaviour. Is the present and very recent mobility of the Earth typical of much or most of the planet's existence, or is it a very late development? Or to put the question in the way it is more usually posed: are plate tectonic concepts applicable to pre-Mesozoic time or not? It is a question every bit as, if not more, fundamental than that raised by Wegener at the beginning of this century.

It is in the nature of things that it should also be a difficult question to answer. What can be said immediately is that if plate tectonics is taken to imply the relative motions of land masses, the extension of the concept to the pre-Mesozoic is inherently implausible. The breakup of the ancient supercontinent of Pangaea some 200 million years or so ago is now well established, and the subsequent motions of the resulting continents have been charted by palaeomagnetic and other means. Finding the physical mechanism for the breakup and subsequent drifting is, of

course, a more difficult matter; but causes apart, the basic idea of continental dispersal from a single land mass poses no severe conceptual problems. By contrast, the proposition that Pangaea itself formed by the amalgamation of many land masses (presumably different in shape and number from the present continents) is conceptually altogether more difficult to accept, and the mechanism for such a union is unimaginable. It would be as easy to accept as, for example, the suggestion that meteorites may accrete in an environment comparable to that in which they disintegrate. That two, or even several, continents may collide and coalesce is not beyond the bounds of comprehension, but that all pre-existing land masses should combine into one can hardly be envisaged. Yet this is precisely what is envisaged by many who have sought to substantiate pre-Mesozoic plate tectonics.

It is at this stage that the dangers of a too uncritical acceptance of some basically uniformitarian viewpoint readily become apparent. It is worth pointing out, for example, that the close association between continental drift (that is, the relative movement of land masses) and plate tectonics at the present time does not necessarily imply that pre-Mesozoic plate tectonic processes would involve drift. In a system of plates in which only the plate containing Pangaea was continental, Pangaea could move relative to the poles as a single land mass; and proof of no relative motion between Pangaea's component masses would not necessarily disprove the existence of plates. This would avoid the problem of the inherent unlikelihood of all land masses coalescing; and the fundamental mobility of the Earth during the pre-Mesozoic need not be rejected.

On the other hand, it must be admitted that the supporters of pre-Mesozoic plate tectonics have often come to that view not directly through a simplistic uniformitarianism requiring the permanence of continental drift, but through an altogether different, though perhaps equally simplistic, uniformitarianism involving pre-Mesozoic drift as a precondition. Specifically, they have argued that modern orogenic belts are the direct result of plate interactions, that the characteristics of ancient orogenic belts are similar to those of more recent ones, and therefore that ancient orogenic belts are the result of ancient plate interactions—in particular the suturing of previously distinct crustal plates which have converged. Africa, for example, contains a pattern of stable cratonic blocks with intervening orogenic belts ranging in age from more than 3,000 million years to Palaeozoic. The argument here then is that the orogenic belts were formed at plate margins, the plates being the cratons which drifted together from positions thousands of kilometres apart.

But although the evidence for this view seems quite strong and is well supported by data from other parts of the world, most notably North America, there is also convincing support for the older, alternative model in which the cratons have remained essentially stationary relative to each other and the orogenic deformation developed between them. There is also a great deal of evidence which at various times has been taken to favour both the stationary and drifting craton models. The fact is that the available data are conflicting; and it seems unlikely that the question will ever be resolved on geological grounds alone. On the other hand, such are the pressures to interpret the past in terms of the present, and such is the strength of the new global tectonic hypotheses, that the case for pre-Mesozoic plate

tectonics has often contrived to appear overwhelming. As recently as last year, for example, McElhinny (*Palaeomagnetism and Plate Tectonics*, Cambridge University Press, 1973) could conclude that it "now seems likely that the processes of plate tectonics have been a feature of all geological time" while at the same time admitting the paucity of palaeomagnetic data from the Precambrian.

That this conclusion may be premature was recently brought home sharply by Piper *et al.* (*Nature*, **245**, 244; 1973) who argued that, whereas the geological evidence may be inconclusive, "palaeomagnetic evidence is potentially decisive". They then concluded from the available palaeomagnetic data that the principal cratonic areas of Africa were probably in roughly their present relative positions as early as 2,200 million years ago and thus that the adjacent orogenies were probably ensialic. More generally, they were able to suggest that the concentration of all existing land masses in one large continent during the late Precambrian remains a serious possibility.

Later in this issue of *Nature*, McElhinny *et al.* present new palaeomagnetic data from Australia and India; and although their interpretation of these and the older data differs from that given by Piper *et al.*, they nevertheless reach the same general conclusions. It thus begins to look more and more as though the inherently implausible never happened. On the other hand, it must be stressed that even now pre-Mesozoic, and especially Precambrian, palaeomagnetic data are still far too sparse to enable any conclusion from them to have the degree of certainty attributed to comparable conclusions relating to the past 200 million years. But what is now certain is that only palaeomagnetism will be able to resolve the issue one way or the other.

P.J.S.

Origin of asteroids

BETWEEN the orbits of the planets Mars and Jupiter resides a collection of minor bodies known as asteroids. They were first discovered in 1801 by the Italian monk Guiseppe Piazzi and caused considerable excitement because their mean solar distance of 2.8 astronomical units fitted neatly into the Titius-Bode series of planetary distances. More than 3,000 have now been discovered and it is estimated that ten times that number may well be susceptible to photographic detection. They are all small, Ceres the largest being about 770 km across, and having a mass of about one five thousandth that of the Earth. In fact the total mass of the asteroids is estimated to be only twice that of Ceres. They also seem to be highly irregular structures, their internal gravitational force being much too weak to make them spherical.

Where do these asteroids come from? Three theories are presently in fashion, the oldest, first propounded by Olbers more than 150 years ago, has the asteroids originating from the cataclysmic disruption of a planet which had an orbit between those of Mars and Jupiter. This received a new lease of life when Ovenden (*Nature*, **239**, 508; 1972), on the basis of his principle of least interaction action, found that he could explain the present planetary positions if another planet ninety times heavier than Earth disappeared between Mars and Jupiter some 16 million years ago.

A second theory (Kuiper, *Astron. J.*, **55**, 164; 1950) produces asteroids by the collisional fragmentation of a small number of planetesimals, these planetesimals being the primaeval condensates from the solar nebula, the fundamental building blocks of the Solar System. The present Hirayama families of asteroids with similar orbital elements are supposedly produced by the fragmentation of individual planetesimals.

Theory three (Alfvén, *Icarus*, **3**, 52 and 57; 1964) has the asteroids accreting by random aggregation. As the bodies are inelastic, collisions tend to reduce relative velocities and thus to equalise orbits. The particles always return to the point in space of their last collision leading to collisional focusing and the production of a stream of particles of very similar orbits and low relative velocities—an ideal situation for accretion. If this theory is correct the asteroids are in the process of growing into a planet with a mass similar to that of Mercury.

Napier and Dodd (Royal Observatory, Edinburgh) in a recent edition of the *Monthly Notices of the Royal Astronomical Society* (**166**, 469; 1974) have tried to discriminate between these theories by considering the observed mass distribution of the asteroids, the observed rotation periods and also the physical tenability of the processes required.

The number-mass distribution of asteroids smaller than 20 km obeys a power law where dN , the number with masses between m and $m + dm$, is given by $dN \propto m^{-8} dm$. Dohnanyi analysing the data obtained from the Palomar-Leiden survey of asteroids finds them to have an s value of 1.84; Hellyer found $s = 1.77 \pm 0.05$ using the same data. Napier and Dodd calculate a theoretical value for s by using a Monte Carlo simulation technique to look at random collisions between particles. Now collisions can cause erosion or fragmentation depending on the size of the incident particle. The authors find that erosion is negligible, a rocky asteroid typically only losing 8% of its mass before it suffers fragmentation. The collision fragments have an s value of between 1.5 and 2.0, the s value increasing with collision energy.

Starting with a sample of 7,400 bodies of differing mass and an s of 1.80, these authors found that 500 random collisions did not change s at all but that after this number the s value slowly increased reaching about 1.90 after 800 collisions. The larger bodies would not obey the power law because they were not being replenished. Napier and Dodd tried a second theoretical model where bodies of the same mass were allowed to collide and coalesce on collision. Independent of whether the accreted particles are replaced s quickly reaches a value of 1.5. These findings indicate that observed asteroidal s values can be produced by fragmentation and also by planetary disruption but not by accretion, thus removing one theory of origin.

Asteroids have rotation periods of around 8 h which for the larger ones is close to the limit for rotation-induced fission. Napier and Dodd find that the observed spin periods can be produced by fragmentation as there is abundant collisional energy available whereas accretion would give unobservably long periods. Fragments from a disrupted planet, however, would have rotation periods similar to that of the planet, which could quite easily have been in the region 8 to 10 h. So once again accretion is ruled out but the other two theories are tenable.

The authors have pointed out (*Nature* **242**, 250; 1973) that Ovenden's hypothetical planet cannot have been disrupted by the release of chemical or nuclear energy or by tidal instability induced by Jupiter. Disruption could be caused by detonation but chemical detonation is of too low an energy while nuclear fission detonation requires the sudden creation of a large supercritical mass within the planet. Another possibility is that the solid planetary mantle constrained a high pressure core until breaking point was reached; however, the tensile strength of rock is such that a planet of mass greater than 4×10^{23} g would not disperse the fragments to infinity and it would be impossible to lose the 99.9% of Ovenden's planet to leave just the asteroids remaining.

So only the planetoid hypothesis is left. This is consistent with meteorite geology which shows evidence of a cooling phase and shock processes which could have occurred at fragmentation. It is temporally reasonable as collision

dynamics indicate that Ceres would collide with one of the 30 asteroids capable of fragmenting it every 10^{10} yr indicating that the large asteroids are original planetesimals. More-

over, the dispersion of fragments after collision can produce orbits of reasonable inclination to the ecliptic just like those of the asteroids.
D.W.H.

Cell movements in *Hydra*

CELL movement is a fundamental component of morphogenesis. Cells may move as three-dimensional masses, in sheets or as individuals. The movements must be controlled by signals available to the cells, and the signals may be involved in the control of development in general. One can therefore deduce much about developmental control from observing cell movements; for example, melanocyte migration in amphibians is under chemotactic control, as is the aggregation of slime mold amoebae.

In *Hydra*, Wolpert has shown that much morphogenesis is possible by a combination of cell movement and differentiation without any increase in cell number. Herlands and Bode (see *Nature*, **248**, 387; 1974) have now published observations on nematocyte migration, which confirm the general model for the control of *Hydra* development put forward by Wolpert and his collaborators.

Nematocytes are stinging cells used for the capture and paralysis of prey. They arise by differentiation from interstitial cells in *Hydra*'s body column, and migrate into the tentacles where they position themselves in the ectoderm. Although there are at least four types of nematocyte, Herlands and Bode examined the two commonest, desmonemes and stenoteles.

The authors grafted the upper halves of *Hydra* labelled with ^3H -thymidine to unlabelled lower halves, and also made the reverse grafts. They examined the originally unlabelled halves for labelled nematocytes. Nematocyte migration was strongly biased apically; indeed, basal migration only occurred towards buds. There was no migration into the peduncle.

As there was thus already a clear suggestion of apical

preference in nematocyte migration, the authors tried further grafting experiments to determine whether the signal was produced by the head or was some underlying property of the cells through which the nematocytes migrate. The authors labelled gastric regions of *H. attenuata* with ^3H -proline and grafted unlabelled heads to either the basal or apical ends. They then measured the rate of accumulation of labelled nematocytes in the unlabelled, grafted heads. They found that stenoteles accumulate at the same rate in basal and apical heads, but that desmonemes, for the first 3 days after grafting, accumulate in apical heads at a greater rate. After 3 days there was an increasing rate of migration by desmonemes into basal heads. These times correlate well with the times for polarity reversal by basally grafted heads found by earlier workers.

This interpretation was checked by grafting a head to the basal end and a peduncle to the apical end of a labelled gastric region. The addition of a peduncle is known to accelerate polarity reversal, and indeed there was no indication that the desmonemes migrated according to the original tissue polarity. Stenoteles, as before, migrated to the head regardless of polarity.

These results show that two kinds of signal are used—both an underlying property of the tissue, which may correspond to Wolpert's "positional value" and which can determine polarity, and a signal emanating from the head independent of polarity. This might be a chemotactic signal. The signals closely correspond to those already invoked for models of the control of *Hydra* development.

From a Correspondent

An SV40 that doesn't help adenovirus

THE inability of human adenoviruses to undergo a productive cycle of growth in simian cells—possibly because of the failure of a late function—can be overcome by coinfection with simian virus 40 (SV40). Adenovirus T-antigen (Feldman *et al.*, *J. Bact.*, **91**, 813; 1966; Malmgren *et al.*, *ibid.*, 262) and viral DNA are produced (Reich *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **55**, 336; 1966; Baum *et al.*, *Virology*, **34**, 373; 1966), whereas the production of some late protein is inhibited in monkey cells (Friedman *et al.*, *J. Virol.*, **5**, 586; 1970; Henry *et al.*, *Nature new Biol.*, **233**, 39; 1971). The region of SV40 DNA that specifies its helper function was located in an adeno-SV40-nondefective hybrid by Lewis and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **63**, 1128; *J. Virol.*, **11**, 655; 1971). These workers selected, from adenovirus populations that had been grown in the presence of SV40, mutants that were able to grow productively on monkey cells without the assistance of exogenous SV40 virus.

Some of these mutants contained segments of SV40 DNA covalently linked to adenovirus DNA sequences. In these adeno-SV40 hybrids, the region of the adenovirus substituted for by the SV40 sequences does not seem to be essential for adenovirus growth. The substitutions of SV40 sequences all begin at the same place in the adenovirus genome (14% from one end) but in different mutants the SV40 sequences can range in amount from 11–43% of the SV40 genome, the smallest being 17% of the genome (see *J. Virol.*, **12**, 643; 1973). All these SV40 sequences start from one place on the SV40 physical map which is 0.11 map units to one side of the *EcoR*₁ cleavage site (see *J. Virol.*, **12**, 653;

1973). The exact nature of the SV40 helper function is not known but it has been mapped in a region of the genome that is thought to specify early functions.

Jerkofsky and Rapp tested the enhancing ability of some temperature sensitive mutants of SV40. Both late mutants, which did synthesise viral DNA, and an early mutant, *tsA7*, which did not synthesise viral DNA, but did induce host DNA synthesis (*J. Virol.*, **8**, 516; 1971), were able to enhance the production of adenovirus at the nonpermissive temperature in monkey cells. These results support their idea (*Virology*, **51**, 466; 1973) that an early function of SV40, possibly related to the induction of host DNA synthesis, is involved.

Kimura, in this issue of *Nature*, has tested the helping ability of temperature sensitive mutants of three complementation groups defined by Kimura and Dulbecco (*Virology*, **52**, 529; 1973). He also finds that late mutants will enhance adenovirus growth at the nonpermissive temperature. The early mutant *ts640* was unable to facilitate adenovirus growth at the nonpermissive temperature although enhancement was normal at the permissive temperature. This mutant *ts640*, like *tsA7*, is temperature sensitive for the production of viral DNA. It is not known whether *ts640* induces host DNA synthesis; it may differ in this respect and thus represent a second class of early mutants of SV40 (possibly a fourth complementation group). A further characterisation of *ts640* should be accomplished soon.

Kimura has mapped his genetic marker in the SV40 helper function: it occurs between 0.11 and 0.28 map units on the SV40 physical map.
From a Correspondent

Wasa's cannon balls saved from corrosion

ON August 10, 1628 the Swedish warship *Wasa* started on her maiden voyage out of Stockholm harbour. She sank within minutes. 333 years later she was raised and is now a magnificent tourist attraction. She is also, to Mr L. Barkman of the Statens Sjöhistoriska Museum in Stockholm, "an enormous corrosion experiment". Barkman, along with Dr O. Arrhenius and Mr E. Sjöstrand report on the problems of preventing further rusting away of iron from *Wasa* in a recent publication of the Korrosionsinstitutet in Stockholm (*Conservation of Old Rusty Iron Objects*, Bulletin 61E, 1973; SKr 16.00).

Even at the time of the *Wasa* mishap the water in Stockholm harbour was fairly noxious. Dumping of rubbish had probably ensured that there was little or no dissolved oxygen present. The water was saline and at a temperature of around 3° C. Into this murky environment dropped a thousand iron objects such as guns, 24-pound cannon balls, chain shot and pike shot. Thousands of nails and one-inch diameter bolts also went down, but of these only two fragments remain. The guns were at first held down in their carriages, but were soon salvaged by the primitive means available in the seventeenth century. Presumably the forgings rusted rapidly—today there is practically no sign of them on the 64 gun carriages and 52 gun ports. The environment is still hostile. New one-inch diameter iron bolts were used during the salvage operation, and after a year the diameter of the worst affected had been reduced to one third of its original value.

While forgings such as nails, bolts and gun fittings disintegrated, cast iron objects fared much better; not that the corrosion was any the less, but castings (cannon balls, for instance) have retained their structure. This is believed to be an effect of the higher carbon (3½%) and silicon (1%) content of the cast-iron balls. Analyses of the rusty outer layer of a typical sample showed that the percentage of carbon and silicon in the corroded material was up to 12% and 5% respectively, whereas iron was down to 43%. Nearly all the iron was in oxides, but there was about 1% chlorine, mostly from chlorides formed in salty water. In a limited-oxygen environment the final product of the oxidation process would be Fe_3O_4 , black magnetite.

The rusted outer layer of cannon balls was on average 1.4 cm thick; this implies a lower corrosion rate than for the bolts, but the majority of balls were protected by mud. It is clear that in the corroded layer many of the iron compounds have been dissolved away but a

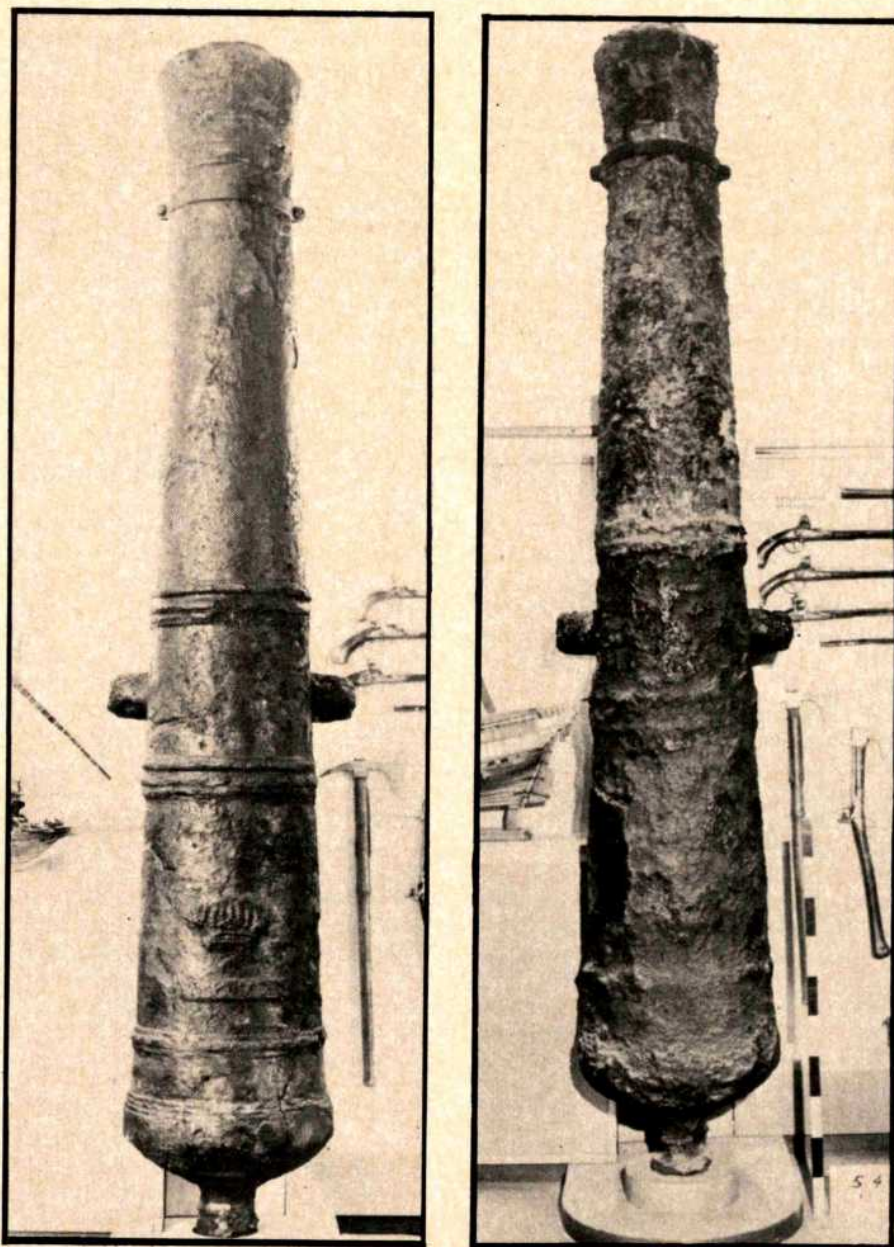
total collapse of the fabric of the rusted layer has probably been averted by the enriched carbon and silicon fraction.

The dissolved iron compounds did not get far, as there were no currents. When *Wasa* was raised it was a rich mixture of colours, from mud mixed with precipitated oxides and salts. Stalactites of iron oxides, silicates and sulphates hung from the decks. The much admired black oak colour of the hull was the result of impregnation with several tons of iron.

A very serious problem arose the moment the iron objects came out of the water. Oxidation continued—now in an oxygen-rich environment so the end product is Fe_2O_3 , red haematite, but more damaging was the chloride activity. In moist air, FeCl_2 and FeCl_3 go through a cyclic process, precipitating iron hydroxide. The pitting that this

causes is the most dangerous threat to iron that has been in seawater, and the threat cannot be arrested by standard cleaning and drying processes. Corrosion in the atmosphere would have rapidly destroyed the museum exhibits. Dramatic evidence of this was already present in Stockholm. A cannon from a seventeenth century wreck had been salvaged in 1953, scraped, brushed, treated with antirust oil and exhibited. The photographs show its decline in 18 years.

Within a month the *Wasa* specimens were visibly corroding further; one cannon-ball fell apart. Unlike most corrosion problems in which the aim is to clear the surface of rust, in this case it was necessary to halt the chemical processes but keep the structure intact. To do this the Stockholm workers decided to attempt to reduce the oxides and



Cannon from the Riksäpplet which foundered in 1676: left, after salvaging in 1953; right, the same with severe corrosion damage in 1972.

chlorides to the metallic form. Hartwich had first done this in the 1880s with small rusty samples at 500 °C in a hydrogen atmosphere; the process is widely used in industry, but not in museums.

A cylinder of depth 126 cm, radius 23 cm was loaded with the samples (for instance six cannon balls) which were then placed in a furnace in hydrogen. Reduction was carried out at 600 to 800 °C and after a period of about a day the process was complete—not only were oxides absent but chlorides too had been reduced to iron. Chemical analyses show that there was generally no trace of chlorine and all the iron was in the metallic form. Subsequent coating with an antirust agent and accelerated rusting tests show that the objects are now fully protected against corrosion. They now sit in the Wasa Museum—uncorroding and with the right shape.

Success of super labs at Cologne meeting

from a Correspondent

THE Spring meeting of the Cologne Institute of Genetics (February 22-24) opened with a symposium on tumour viruses which, as W. Doerfler (Cologne Institute of Genetics)—who organised it—pointed out, really dealt with DNA viruses, since even the RNA viruses discussed by P. Vogt (University of Southern California) and J. Dahlberg (University of Wisconsin) have a transitional DNA phase.

Transcriptional maps of the adenovirus genome in productive infection are emerging both from the Wallenberg Laboratory at Uppsala and from Cold Spring Harbor. L. Philipson (Wallenberg Laboratory) described the techniques used by his group to obtain adenovirus DNA fragments from cleavage with restriction enzyme and labelled either in the heavy or the light chain. The amount of labelled DNA could be measured after the templates had been hybridised with excess unlabelled RNA, to give the quantity of DNA transcribed from either the light or the heavy strand of the restriction enzyme fragments.

Philipson's results proved entirely compatible with those of the Cold Spring Harbor group, presented at the symposium by W. Keller. The highlights of Keller's talk were the description of a new approach to sequencing viral DNA using a series of restriction enzyme cleavages which should greatly facilitate what is normally a long and painful process, and the results of the analyses of six hamster cell lines transformed with adenoviruses.

All the transformed lines seem to

hand, at least a portion of restriction fragment F located near the middle of the DNA towards the C fragment end was absent from all the lines. Keller speculated that the adenovirus DNA was integrated into the host chromosomes and that the functions contained in the retained portion of fragment A were necessary for its maintenance.

A. Lewis (National Institutes of Health), in a thorough analysis of the non-defective adenovirus-SV40 hybrids, described the unequivocal mapping by heteroduplex analyses of the order of several early SV40 functions. The question whether viral DNA becomes integrated into 'transformed' lymphocytes was raised by T. Lindahl (Karolinska Institute), who showed that the link between Epstein-Barr virus DNA to lymphoblast DNA was not stable in alkali, although a substantial portion of the viral DNA banded in caesium chloride with host DNA. Only when the DNA was sheared into small fragments were the host and viral DNA sequences effectively separated by isopycnic banding.

Vogt effectively summarised the work on RNA tumour viruses with a lucid exposition of the dilemmas with which analyses of the structure of oncornavirus are fraught. Most of the data, including those on the kinetic complexity of the genome, suggest that it is haploid and segmented. But reservations on this conclusion must remain because all of the RNA segments, and not just one, decrease in size on the mutation of sarcoma viruses to become defective or leukaemogenic.

The symposium concluded with a *tour de force*: the announcement of the sequence of the transfer RNA molecule which serves as the primer for DNA synthesised on the 70S RNA template by reverse transcriptase. This was the fruit of a collaborative project involving Dahlberg at Madison (who presented the paper) and M. Bishop's laboratory at the University of California in San Francisco, and it illustrated one of the most conspicuous incidental points brought out by the meeting. This was the contrast in the accomplishments of the large institutionalised collaborative groups such as those at Uppsala, Cold Spring Harbor, NIH, and on the San Francisco-Madison axis, and those of the smaller laboratories, both European and American, represented at the meeting. It was the superlabs which, by approaching complex problems from several directions simultaneously, emerged as the more effective, despite the inevitable loss of scientific identity resulting from the dilution of credit among individual contributions.

have a portion of the terminal restriction fragment A and all but one contain at least a portion of the C fragment located at the other end. On the other

Perfusing the female reproductive system

from our
Steroid Biochemistry Correspondent

WITH the well known limitations of most *in vitro* techniques for studying the metabolism of tissues, the use of perfusion or superfusion techniques has become more popular. In the field of reproductive biology, interesting results have been obtained by perfusion of the human ovary, placenta or foeto-placental unit. Tojo *et al.* (*Amer. J. Obstet. Gynec.*, 118, 119-129; 1974) have now achieved *in vitro* perfusion of the human uterus and fallopian tubes as well as the ovary.

Before removing the utero-tubal-ovarian unit, the arterial pressure, pulse rate and blood flow were measured and the pressure wave form of the uterine arteries was recorded. The unit was connected to the perfusing machine through the bilateral uterine arteries and the bilateral uterine and ovarian veins and was perfused in such a way that the pulse rate and pressure of the input of the perfusate were similar to those expected in the uterine artery *in vivo*. This was achieved by using a Bellofram-type artificial heart driven by a liquid amplifier, a pressure wave form regulator for the perfusate, an oxygenator, organ chamber, suction pump for venous return and an autoregulatory thermocontrol mechanism.

The viability of the unit during perfusion was tested by measuring the erythrocyte count, haematocrit, pO_2 , pCO_2 and pH values and the haemoglobin, lactate and pyruvate content of the perfusate.

Tojo *et al.* obtained successful perfusions of three utero-tubal-ovarian units, one obtained during the menstrual period and two during the mid-luteal phase of women with normal ovarian function. Addition of human chorionic gonadotrophin (HCG) to the perfusate increased the progesterone secretion from the ovary obtained during the luteal phase but not from that obtained during menstruation. When tissues obtained at the thirteenth week of pregnancy were perfused, there was a rapid increase in the HCG content and a gradual increase in the content of human placental lactogen (HPL) of the perfusate. Active nucleic acid synthesis was shown to occur in the trophoblastic tissue.

These preliminary results suggest that the utero-tubal-ovarian unit maintains its viability for some considerable time when perfused. If this is confirmed in further investigations, application of this method should help in the elucidation of many problems of reproductive physiology and pathology.

Rethinking about the sarcoplasmic reticulum

from a Correspondent

COINCIDENTALLY independent groups in London, Toronto and Heidelberg, have published in three different journals results that clarify the disposition and functions of membrane proteins in the sarcoplasmic reticulum of rabbit skeletal muscle.

Major proteins

The sarcoplasmic reticulum is a membrane specialised for transport and binding of calcium in muscle tissue and, as it has few proteins, it presents considerable advantages for study of cation transport through membranes. The major protein is a Ca^{2+} -activated ATPase (molecular weight approximately 102,000-115,000) which is tightly integrated into the membrane and is only released by detergents. Several other acidic proteins can be removed more easily, by extraction with chelating agents for instance. Calsequestrin, a protein with the ability to bind large numbers (30-40 per mole) of calcium ions at rather low affinity in the presence of 0.1M KCl, was originally suggested by MacLennan to play a part in cation binding in the reticulum. Calsequestrin is a glycoprotein and contains about 1 mol. of sialic acid, clearly not enough to account for the total number of cation binding sites, most of which probably involve acidic amino acid residues of the protein.

Another acidic protein, with molecular weight 55,000, binds calcium ions with high affinity even at high ionic strength (0.1M KCl) but with low capacity (about 1 mol. per mol). The other acidic proteins bind calcium but with very low affinity at any ionic strength. There is, however, some genetic variability in the total number of high or moderately high affinity calcium binding proteins and certain rabbits contain a second calsequestrin that is slightly smaller, with molecular weight 44,000 (MacLennan, *J. biol. Chem.*, **249**, 980-984; 1974). The mutation in this membrane protein seems to be a deletion and loss of a peptide sequence rich in methionine and cysteine. The mutant sarcoplasmic reticulum seems to be more active in Ca^{2+} transport than normal sarcoplasmic reticulum and the Ca^{2+} binding ability of the mutant protein is not affected by the deletion.

Calsequestrin

Calsequestrin was believed originally to be located in the interior of the sarcoplasmic reticulum and to play a part in sequestering calcium transported to

the inside of the vesicle by the Ca^{2+} -activated ATPase. Thorley-Lawson and Green (*Eur. J. Biochem.*, **40**, 403-413; 1973) suggest, however, that calsequestrin may be at the exterior face of the sarcoplasmic reticulum membranes, in sites exposed to the aqueous environment. This is certainly a more likely location for an extrinsic protein such as calsequestrin, which can be largely removed from intact closed membrane systems by extraction with 1 mM EDTA. The material removed by the chelating agent is reversibly bound to depleted membranes in the presence of Ca^{2+} indicating a binding to the lipid bilayer mediated by cations, with Ca^{2+} perhaps involved in some interaction between the acidic calsequestrin protein and the polar head groups of phospholipids. Candidates for a function as an internal calcium store may therefore have to be looked for elsewhere; possibly the acid protein of molecular weight 55,000 fulfils this role.

Calcium-activated ATPase

A second revision of the original model proposed by MacLennan for membrane proteins in the sarcoplasmic reticulum concerns Ca^{2+} -activated ATPase. These molecules were thought to be entirely represented by the 7.5-9 nm diameter particles seen in the freeze fractured faces of the membrane, that is to say, placing the ATPase molecules within the hydrophobic interior of the membrane. The recent reinterpretation makes the more acceptable proposition that the particles revealed in freeze fractured preparations represent the hydrophobic 'tail' of the ATPase that is firmly integrated into the membrane interior. The density of intramembranous protein domains is similar to that found in human erythrocyte membranes and representing the hydrophobic 'tail' of the major glycoprotein. About half of the Ca^{2+} -activated ATPase molecule is exposed on the exterior surface of the lipid bilayer where it is available for reaction with trypsin or lactoperoxidase (Thorley-Lawson and Green, *loc. cit.*; Migala, Agostini and Hasselbach, *Z. Naturf.*, **28**, 178-182; 1973; Stewart and MacLennan, *J. biol. Chem.*, **249**, 985-993; 1974). Since the sarcoplasmic reticulum is impermeable to protein reagents, trypsin and lactoperoxidase will initially only modify proteins on the external surface of these microsomes.

Structural relationships

The ATPase forms projections which consist of a 4 nm knob on a 2 nm stalk extending outwards from the external face of sarcoplasmic reticulum and seen in negatively stained preparations.

Very similar projections are produced in vesicles formed artificially from purified ATPase by removal of detergent from solubilised preparations of the enzyme. The projections are destroyed by high concentrations of trypsin with loss of enzymatic activity. ATP (and sucrose) protects the loss to some extent. Thorley-Lawson and Green (1973) found that at lower levels of trypsin, ATPase is split into two pieces of very similar size (40,000 and 55,000) which remain attached to the membranes. The fragments of molecular weight 60,000 are degraded to yet smaller units of molecular weights 33,000 (that contains the phosphorylated site) and 24,000, and all of these are iodinated by lactoperoxidase added externally suggesting that they exist on the outer surface of the membrane. The fragment of molecular weight 60,000 thus represents the stalk of the ATPase and the 55,000 molecular weight fragment, which is not iodinated, is buried within the membrane, as expected for a hydrophobic 'tail' equivalent to particles of the size seen in the freeze fractured faces of the internal membrane. The internal particles and the non-iodinated fragments of molecular weight 55,000 persist within the membrane of extensively trypsinised preparations which is consistent with these being the part of the ATPase integrated into the membrane. Thorley-Lawson and Green found that this part of the enzyme molecule is not iodinated even when both faces of the membrane are accessible to labelling reagents. If the ATPase does span the width of the membrane therefore, and this is the simplest model to explain the Ca^{2+} transfer across the membrane, it does not expose any significant amounts of iodinated tyrosine nor trypsin sensitive bonds in the region.

The structural relationship of the intramembranous particles and Ca^{2+} -activated ATPase is also implied by their coordinate increase in the muscle microsomes during chick embryonic development. Two papers by Martonosi and his colleagues (*J. biol. Chem.*, **249**, 612-623 and 624-633; 1974) describe developmental changes in the sarcoplasmic reticulum of chick skeletal muscle. The increased transport of Ca^{2+} during embryonic and postnatal development is directly related to concentration of Ca^{2+} -activated ATPase in the membranes of the sarcoplasmic reticulum. There seems to be an inverse relationship during embryogenesis between the appearance of the Ca^{2+} -activated ATPase and a Ca^{2+} -insensitive Mg^{2+} -activated ATPase. This second enzyme appears early in development and subsequently declines. It would be interesting to know whether these ATPases are independently controlled. The alternative is to assume a precursor

function for the Mg^{2+} -activated enzyme which acquires calcium sensitivity at a relatively late stage in development, for example, by interaction with 'coupling factors' which link ATP hydrolysis to Ca^{2+} transport. The lack of a subunit structure for the Ca^{2+} -activated ATPase of rabbit sarcoplasmic reticulum tends to make this assumption less likely, although it is an intriguing possibility.

Solar wind blows gusty at sunspot maximum

by our Cosmology Correspondent

THE continuing story of the influence of the Sun on terrestrial events has developed a stage further with the report by Intriligator of observations which provide the first evidence for long term variations in the solar wind associated with changes in the solar cycle (*Astrophys. J. Lett.*, **188**, L23; 1974). Events such as weather variations and modulation of the Earth's spin rate associated with the solar cycle of activity are now becoming well documented, and in recent years it has been established that there is a solar modulation of cosmic rays which is in anti-phase with the solar cycle. All this evidence points to variations in the solar wind as, if not the root cause, then at least the next link in the causal chain. But since suitable space vehicles which can provide essentially continuous monitoring of the solar wind have only been available for some ten years—about the length of one solar cycle—it is hardly surprising that such solar wind variations have only now been directly confirmed.

Intriligator's work is concerned with variations in the solar wind which are the result of changes in the high speed stream structure. The data were obtained by Pioneer and Vela spacecraft; they show variations in the number and intensity of high speed proton streams, and the average solar wind speed, between 1965 and 1971. Early in the period studied a high solar

wind speed of 626 km s^{-1} was measured, on January 20, 1966, and in all five high speed streams (the slowest with velocity 497 km s^{-1}) were observed between December 25, 1965 and February 3, 1966. But this represented the low point of activity during the period of the investigation.

When longer stretches of data are examined, it turns out that there were more high speed streams in 1968 (44) than in any of the other years of the study; as Intriligator puts it, the solar wind is more "gusty" around the time of maximum solar activity. On 230 days in 1968 there was high speed streaming activity in the solar wind; at the other extreme, all but 73 days out of 270 studied in 1971 were free from gusts.

This immediately offers an explanation of the modulation of cosmic rays over the solar cycle. At solar maximum, there is a much greater chance that cosmic rays entering the Solar System will encounter high speed streams, and this produces more modulation of the cosmic rays. When the solar wind is calm, it is easier for cosmic rays to penetrate the solar wind and reach Earth. The yearly average of the solar wind speed itself varies over the solar cycle, and is highest at solar maximum; there seems little doubt that this is a real effect which provides an important insight into the workings of the Solar System.

Genome organisation in eukaryotes

from a Correspondent

ANALYSIS of the organisation of nucleotide sequences in DNA of the more highly evolved eukaryotes is proceeding rapidly at present and has revealed some intriguing but so far unexplained phenomena. Following up their initial observations on the formation of DNA rings and other circular structures (*J. molec. Biol.*, **51**, 621; 1970), Thomas and his associates in a series of papers (*ibid.*, **77**, 25-99; 1973) have estimated that as much as half the DNA in mammals, insects and amphibians is composed of tandemly repeated or intermittent tandemly repeated sequences clustered in groups which they call *g* regions. The number of *g* regions in three *Drosophila* species is about 5,000 which corresponds to the number of cytologically observable bands or chromomeres in their polytene chromosomes.

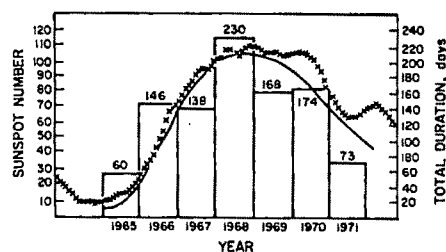
Similar measurements for the mouse and *Necturus*, which have respectively fifteen and seven hundred times more DNA than *Drosophila*, reveals that the number of *g* regions is about 40,000 and 1.5 million respectively. This probably means that the one to one correlation between *g* region and

chromomere number observed in *Drosophila* does not hold in other organisms since the chromomere number (lampbrush chromosome loops) in *Triturus* has been estimated at 4,000. These results have relevance for the problem of the very large differences in genome size between closely related organisms. Increased genome sizes seem to be based largely on increased numbers and only marginally on increased size of *g* regions, since the estimated sizes of *g* regions in mouse and *Necturus* are only twice that of *Drosophila*.

The kinds of experiments Thomas and his colleagues have done to reach these conclusions are the measurement of the frequency and contour length of rings and ringlike structures observed by electron microscopy in preparations of DNA renatured to low C_0t values after treatment with *Escherichia coli* exonuclease III, an enzyme which digests one strand of a DNA duplex from 3' to 5'. DNA molecules resected by this enzyme will have two single strand ends which if complementary will tend to renature to give rings and other ring-like structures. No rings are produced by treatment of bacterial DNA in the same way, though many phages may cyclise through the presence of so called sticky ends which may occur naturally as in λ or produced by exonuclease III treatment as in T7.

Thomas *et al.* have shown that the frequency of rings is linearly related to the extent of resection up to a maximum plateau level which is 300 nucleotides for mouse and *Drosophila* and 500 for *Necturus*. These figures are presumably related to the length of the repeating sequences involved. Providing rings are formed in optimal resection and renaturation conditions, the frequency of rings is primarily a function of DNA size and is maximum with DNA of 1-2 μm in length regardless of organism. Such rings have very good thermal stability suggesting that the region of overlap is of the order of 200 nucleotides with very little if any mismatching, since thermal stability under ionic conditions reflects both length and fidelity of hybrid duplex. This means that the minimum length of the repeat unit is 200 nucleotides and that they show very little divergence by base substitution.

Fewer rings are created both with larger and with smaller DNA molecules. The reduction with increasing length is interpreted to mean that related repeated sequences are clustered into regions which Thomas *et al.* call *g* regions. An alternative explanation for this observation—that longer fragments have a greater distance separating potentially reactive single strand ends resulting in slower renaturation kinetics—was eliminated by a study of the effect of fragment length on the rate of



Total number of days on which high speed "gusts" were observed in the solar wind. —, Smoothed sunspot numbers; —x—, measured sunspot numbers. Data for 1965 and 1971 are based on 136 and 270 days of data, respectively.

cyclisation. Similarly the reduction with small DNA molecules was not due to slower renaturation kinetics of small fragments resulting from their decreased flexibility. It is argued that reduced ring frequency with molecules less than 1-2 μ m is evidence for tandem repeats being spaced by non-repetitious sequences. Although Bick, Huang and Thomas (*J. molec. Biol.*, **77**, 75; 1973) tried to find evidence for the presence of this predicted non-repetitious DNA by examining rings prepared for electron microscopy in high concentrations of formamide, which would reveal unpaired single stranded regions within the terminal region of overlap, they were unable to do so. They concluded that either the non-repetitious sequences do not exist, which is unlikely, or they are too long or too short to be observed.

Similar conclusions concerning the interspersion of repetitive and non-repetitive sequences have been reached by other groups using different techniques. For instance, Davidson *et al.* (*J. molec. Biol.*, **77**, 1-23; 1973) used labelled *Xenopus* DNA sheared to various known lengths denatured and then renatured, in the presence of excess cold *Xenopus* DNA of 450 nucleotides length, to C_0t 50, at which point predominantly repetitive sequences have renatured. They found that as the length of labelled DNA increases so a greater amount is bound to hydroxyapatite. Hydroxyapatite binds double stranded but not single stranded DNA at low salt concentrations and therefore allows their separation. In the conditions of the experiment any non-repetitive sequences, although remaining single stranded, will bind to hydroxyapatite if they are covalently linked to renatured repetitive sequences.

The curve relating the proportion of 3H -labelled DNA fragments containing a repetitive element to fragment length shows three things. A steep linear increase (4% per 100 nucleotides) up to an inflexion point at about 700 nucleotides length and 60% fragments bound, followed by a slower linear increase (0.7% per 100 nucleotides) up to 4,000 nucleotides length and 80% fragments bound. The first part of the curve does not extrapolate through zero bound at zero length but through about 20% representing the amount of repetitive DNA in the genome. Davidson *et al.* interpret these data on the basis of a model in which 50% of the *Xenopus* genome consists of repetitive elements about 300 nucleotides long interspersed with non-repetitive sequences 700-900 nucleotides long. A further 25% consists of repetitive sequences interspersed with longer (>4,000 nucleotides) non-repetitive sequences. The remainder is composed of unique sequences (20%) without detectable repetitive elements and of tandem, low complexity repeti-

tive sequences (5%) without detectable non-repetitive elements. Similar experiments with sea urchin DNA (Graham *et al.*, *Cell*, **1**, 127; 1974) show that an identical model may be constructed. Furthermore, in two earlier papers (Kram *et al.*, *J. molec. Biol.*, **64**, 103; 1972; Wu *et al.*, *ibid.*, **64**, 211; 1972) on the organisation of repetitive sequences in *Drosophila* DNA other groups reached similar conclusions.

Although it seems likely on present evidence that a general model in which repeated sequences alternate with unique sequences in eukaryotic DNA can be constructed, a good deal more work is required to reveal in detail which repeating patterns are universal and to provide some notion of their relevance to genome function.

Pattern formation in insects

from our Insect Physiology Correspondent

THERE are two ways in which the differentiated pattern of the living body may be supposed to arise. Each group of cells forming a given element in the body pattern may be derived from one progenitor cell, so that each component of the pattern represents a clone of cells which continues to propagate a persisting state of determination. Or, the cells forming an element of the body pattern may not have a different ancestry, or 'lineage', from the surrounding cells: their characters may have been determined by their position in the body. It can, of course, be argued that the second alternative is merely a repetition later in development of the same process by which the initial clones of the former alternative came into existence.

In normal development it seems clear that both these modes are concerned in the final generation of pattern. Many years ago, Sturtevant utilised the tendency of certain strains of *Drosophila* to form sexual mosaics (as the result of a spontaneous elimination of an X chromosome from somatic cells during embryonic development) in order to demonstrate the stage of development at which this mutation occurs and to relate this with the mosaic pattern in the resulting adult. This was done by coupling the mosaic strain with bristle characters that could be observed in the cuticle surface, and using the results to study the derivation or 'cell lineage' of the surface of the adult fly. Sturtevant found that related cells remain adjacent in patches without intermingling; that the occasional loss of an X chromosome happens at the early cleavage divisions; and that the mosaic patches of the opposite sex represent clones of these mutant cells.

Recently there has been a renewal of

interest in this approach to the problem of differentiation. Garcia-Bellido *et al.* (*Nature new Biol.*, **245**, 251; 1973) used somatic crossing-over induced by X rays to generate marked clones in *Drosophila* in which he could follow the distribution of characters in the cuticle of the wing surface. The resultant wings were made up of mixed clones, some of marked cells homozygous for the wild type allele of minute, M^+/M^+ ; others, the unmarked cells of the heterozygous strain, M^+/M^- . The marked cells have a much higher growth rate than the unmarked heterozygote. But in spite of this they did not overgrow the unmarked cells. It turned out that the wing is subdivided into eight compartments, and, provided a clone is generated after the time of final subdivision, it is strictly confined to one compartment. But within this compartment the cells could be differentiated by local control so as to contribute to the ordinary wing surface, to parts of wing veins, or to marginal bristles—depending not on cell lineage but on position at the time of differentiation.

Lawrence (*J. Embryol. exp. Morph.*, **30**, 681; 1973) now describes experiments in this same general field carried out on the milkweed bug *Oncopeltus*. He deals solely with the integument of the abdomen. Mild X irradiation of the egg led to somatic mutations which resulted in changes in the pterin pigments of the epidermal cells from the normal orange to deep orange, transparent, white or pink. As in Sturtevant's experiments these mutations resulted in clonal patterns of epidermal cells which are readily recognised by their unusual pigmentation. The results provide much information about the timing of determination for the structure of the abdomen, the number of cells which furnish the ancestry for a single abdominal segment and so forth. Again it is evident that the clones are subject to strict control. If the ancestral cell mutates after segmentation of the presumptive abdomen has been determined (during blastoderm formation), the clones are restricted to a single quadrant of a segment (dorsal or ventral, left or right). The daughter cells forming the members of a clone tend to hold together in a clump, but they can mix with their neighbours and the groups become fractionated to some extent. On the other hand, they cannot cross the boundaries between segments, between dorsal and ventral, or across the midline of the body.

The effective boundaries between segments are established at the time, 10-24 h after egg laying, when the limiting membranes of the cells are developing in the blastoderm. The nature of these and other invisible boundaries between compartments will be a key question in the understanding of differentiation.

Palaeomagnetic results and late Precambrian glaciations

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A combined late Precambrian to early Palaeozoic polar wander path, which differs from that previously proposed for Africa and South America, is derived for Gondwanaland. It suggests that Gondwanaland existed at least 750 Myr ago. The widespread distribution of late Precambrian glaciations can be explained in terms of this polar migration. It is predicted that the late Precambrian and Cambrian part of the North American polar wander path may be more complicated than previously supposed.

PIPER *et al.*¹ have tested whether the concepts of modern plate tectonic theory, as applied to several Phanerozoic orogenic belts²⁻⁶, can be applied to all ancient orogenic belts. Their analysis shows that all palaeomagnetic pole positions for Africa between 2,200 Myr ago and about 450 Myr ago (close of the Ordovician) lie on a single apparent polar wander path, irrespective of cratonic region. This suggests that the major cratonic areas were approximately in their present relative positions and orientations as early as 2,200 Myr ago. The younger intervening orogenic belts did not, therefore, result from plate accretion and subduction processes during an episode of major ocean closure which culminated in a continent-continent collision^{7,8}. Rather, the data support the view that these belts formed *in situ*, the stable cratonic nuclei remaining in place while the intervening belts themselves were being reactivated^{9,10}. Present data cannot, however, preclude the possibility that the belts resulted from the successive opening and closing of small intercratonic oceans.

Comparing with American Precambrian data, Piper *et al.*¹ further suggest that South America and, until about 1,000 Myr ago, North America may have been joined to Africa, and the concentration of all continental crust in one large mass until that time remains as a possibility. A further consequence of their interpretation is that if any of the Precambrian glaciations which affected south-west, central and north-west Africa, and parts of South America, originated between 900 and about 650 Myr ago, then they were formed in low latitudes^{11,12}.

New results from late Precambrian and early Palaeozoic formations in Australia (ref. 13 and J. W. Giddings and B. J. J. Embleton, unpublished information) and India^{14,15} allow us to extend the comparisons of Piper *et al.*¹ to include the whole of Gondwanaland. Our results lead to a different interpretation of the late Precambrian to early Palaeozoic apparent polar wander path. A consequence of this is that late Precambrian glaciations are restricted to high latitudes, as may be expected. Our interpretation does not, however, affect the general conclusions of Piper *et al.*¹, but leads to some predictions concerning the apparent polar wander path for North America.

Gondwanic palaeomagnetism

Palaeozoic and Mesozoic palaeomagnetic results, when referred to the reconstruction of Smith and Hallam¹⁶, suggest

that a common apparent polar wander path can be applied to the supercontinent from some time in the early Palaeozoic until its break up in the Mesozoic¹⁷. Interpretation of the early Palaeozoic section of the path has been uncertain because the poles tend to form rather a loose group in the region off north-west Africa. Stratigraphic sequences have been studied in both northern Australia and the Amadeus Basin of central Australia^{13,18}. The studied strata range in age from Precambrian-Cambrian through to the Ordovician. In each of these unrelated sequences a very large polar shift of 50°-60° of arc is observed during the earliest Cambrian, and in the Amadeus Basin¹⁸ the total shift during the early-Palaeozoic is about 90°. Figure 1 compares the Australian results on the reconstruction of Gondwanaland, with the corresponding late Precambrian and early Palaeozoic path for Africa and South America¹. It can be seen that the two Cambrian sections of the curves do not agree, and in fact have polar shifts in opposite directions.

There are two possible explanations of this disagreement. First, is that the earliest Cambrian sections of the two paths could refer to separate plates which collided to form Gond-

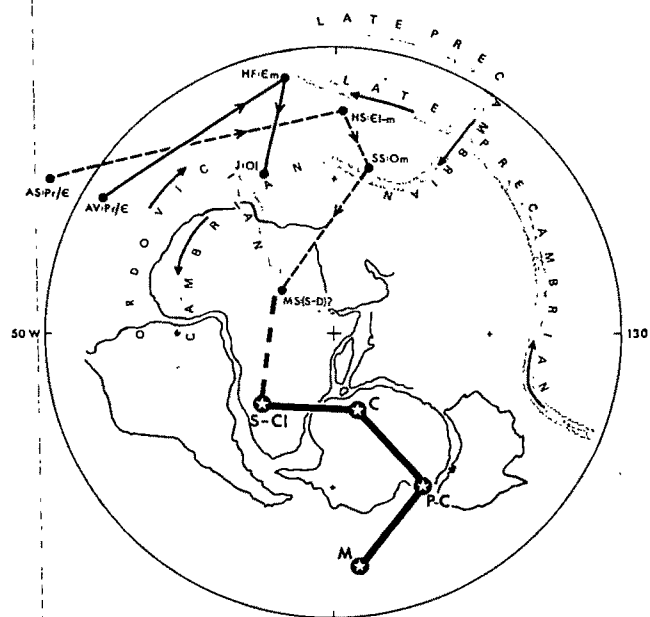


FIG. 1 Late Precambrian to early Palaeozoic apparent polar wander path for Africa and South America suggested by Piper *et al.*¹ (stippled path), plotted against the reconstruction of Smith and Hallam¹⁶ with Africa in its present day position. The large Cambrian polar shift observed in two stratigraphic sequences in northern Australia (solid line) and central Australia (dashed line) are shown for comparison. The pole designations are as listed in Table 1. Note that the path of Piper *et al.* places Africa in equatorial latitudes during the latest Precambrian. The solid black line connecting the mean Silurian-Lower Carboniferous (S-Cl), Carboniferous (C), Permo-Carboniferous (P-C) and Mesozoic (M) poles is the common path for this time interval^{13,17}.

TABLE 1 Gondwanic continents: late Precambrian-early Palaeozoic pole positions

Rock unit	Symbol	Age (Myr)	Palaeomagnetic Pole coordinates	
Africa				
Bukoban Sandstone	BS	Pr(~ 1000)	40°N	317°E
Kigonero Flags	KF	Pr(> 890)	12°N	273°E
Kleinkaras Dykes	KK	Pr(878 ± 41)	20°N	294°E
Gagwe Lavas	G	Pr(813 ± 30)	29°S	293°E
Bukoban Intrusives	BI	Pr(806 ± 30)	11°S	281°E
Mbozi Complex	M	Pr(743 ± 30)	72°S	248°E
Lower Buanji Series	LB	($< 1,350$)	87°S	83°E
Pre-Nama Dykes	ND	Pr(653 ± 70)	85°S	48°E
Plateau Series, Zambia (i)	PZA	($< 1,000$)	71°S	353°E
Plateau Series, Zambia (ii)	PZB	($< 1,000$)	60°N	25°E
Sijarira Group	SG	Pr/€	2°N	352°E
Ntonya Ring Structure	N	Pr(630 ± 24)	28°N	345°E
Klipheuvelformation	K	Pr/€1	16°N	316°E
Ouarzazate Volcanics	OV	Pr/€	30°N	237°E
Amouslék Tuffs	AT	€1	41°N	250°E
Sabaloka Ring Structure	SR	(> 540)	83°N	339°E
Fish River Series	FR	€1	55°S	317°E
Moroccan Lavas	ML	€m	53°N	34°E
Table Mountain Series	TM	0	50°N	349°E
Hook Intrusives	HI	01(500 ± 17)	14°N	336°E
Plateau Series, Zambia (iii)	PZC	Lr.Pal	10°S	352°E
Plateau Series, Zambia (iv)	PZD	Lr.Pal	22°N	19°E
Antarctica				
Charnockites	C	€u-01	2°S	20°E
Sør Rondane Intrusives	SRI	01-m(485 ± 25)	28°S	10°E
Australia				
Precambrian Dykes, B Group	B	Pr(750)*	24°S	282°E
Pound Quartzite	PQ	Latest Pr	60°S	6°E
Antrim Plateau Volcanics	AV	Pr/€1	9°S	340°E
Arumbera Sandstone	AS	Pr/€1	8°N	325°E
Aroona Dam Sediments	AD	$> €1$	36°S	33°E
Hugh River Shale	HS	€1-m	11°N	37°E
Hudson Formation	HF	€m	18°N	19°E
Lower Lake Frome Group (Flinders Ranges)	FRS	€m	8°S	25°E
Upper Lake Frome Group (Balcornacana Creek)	BC	€u	38°S	26°E
Jinduckin Formation	J	01	13°S	25°E
Stairway Sandstone	SS	Om	2°S	50°E
Mereenie Sandstone	MS	(S-D)?	41°S	40°E
India				
Malani Rhyolites	MR	Pr(745 ± 10)	42°S	115°E
Bhander Sandstone	BH	Pr/€	49°S	33°E
Upper Rewa Sandstone	UR	Pr/€	35°S	42°E
Upper Bhander Sandstone	UB	Pr/€	32°S	19°E
Purple Sandstone	PS	€1	28°S	32°E
Salt Pseudomorph Beds	SP	€m	27°S	33°E
South America				
Purmamarca Village	PV	€	61°N	293°E
South Tilcara	ST	€	52°N	27°E
North Tilcara	NT	€	49°N	24°E
Purmamarca	P	€	5°N	39°E
Abra de Cajas	AC	€	2°N	28°E
Salta and Jujuy	SJ	€-0	12°N	329°E
Salta	S	0	31°N	13°E
Sediments, Bolivia	SB	0	4°N	302°E
Urucum Formation	UF	0-S	17°N	347°E

Age symbols: *provisional Rb-Sr age; Pr, Precambrian; €, Cambrian; 0, Ordovician; S, Silurian; D, Devonian. Upper, Middle and Lower divisions denoted by u, m and l respectively.

References: Africa^{1,17,21}; Antarctica¹⁷; Australia¹³, McElhinny, M. W., Giddings, J. W., and Embleton, B. J. J., not yet published; India^{14,15,17,19}; South America^{17,20}.

wanaland during the mid-Cambrian. The timing then corresponds to the peak of the 550 ± 100 Myr pan-African orogeny, which was not the result of plate convergence¹. It thus seems unlikely that any one of these orogenic belts, such as the Mozambique belt, represents the suture between an eastern and a western Gondwanaland. The alternative is that it may be possible to re-interpret the African and South American data to conform with the Australian results.

Pole path for Gondwanaland

Table 1 lists all the palaeomagnetic poles for the Gondwanic continents between about 1,000 Myr and about 450 Myr (close of the Ordovician).

All the palaeomagnetic poles of Table 1 have been analysed with respect to the reconstruction of Gondwanaland of Smith

and Hallam¹⁶, and are shown in Fig. 2. We have been able to interpret all the data to account for the Cambrian polar shift observed in the stratigraphic sequences in Australia. All the South American poles designated Cambrian by Thompson²⁰ now fall on the Cambrian section of our combined path. African poles younger than about 700 Myr, but pre-Ordovician, have very poor age control, with the single exception of the 630 ± 24 Myr Ntonya Ring Structure of Malawi. We have been able to incorporate these poles into our combined path by minor rearrangement of their sequence without violating the tolerances on their age control. Significantly, this places the Ntonya pole at a point on the path just before the Precambrian-Cambrian boundary, whereas in the African path proposed by Piper *et al.*¹ it was out of sequence and inferred to have a much younger age.

We have not drawn the path through the two late-

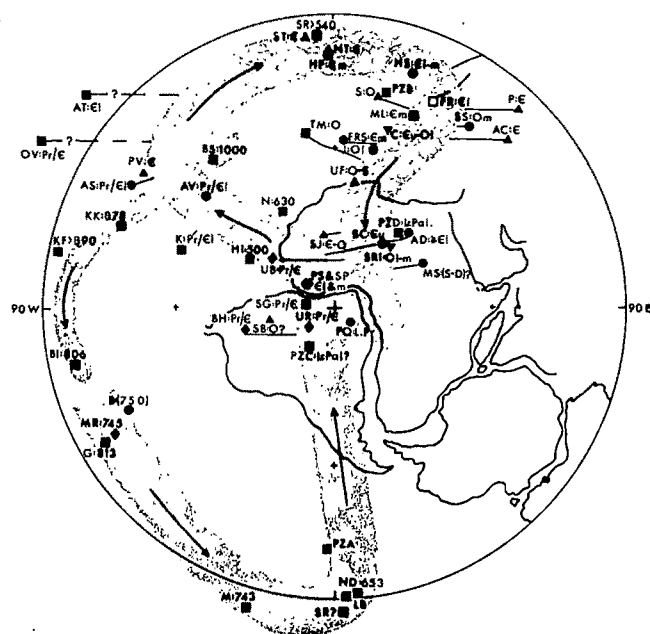


FIG. 2 Revised late Precambrian to early Palaeozoic polar wander path using all the Gondwanic poles listed in Table 1. Bearing in mind the uncertainties in some of the ages and the errors associated with the pole positions, all poles younger than 750–800 Myr are consistent with a common polar wander path when referred to the Smith–Hallam reconstruction¹⁶. The path is indicated by the broad band drawn through the poles. Before 750–800 Myr the sequence of poles is as yet only defined by African data indicated by the narrower band going back to about 1,000 Myr. ■, Africa; ▼, Antarctica; ●, Australia; ◆, India; ▲, S. America. Labelling as in Table 1.

Precambrian and early Cambrian Moroccan poles²¹ because this part of Africa might be a remnant of a Precambrian North America. It would not, however, be difficult to incorporate these into a slightly modified path, and then also identify a large Cambrian shift for North Africa to the pole for the Middle Cambrian Moroccan lavas. Latest Precambrian poles cluster in the region west or south of north-west Africa. The pole from the Cambro-Ordovician Hook Intrusives of Zambia is in this group but its circle of confidence is large enough for it to be associated with the later part of the curve in the region of the Sahara. The Cambrian poles from the Salt Range also seem to be out of sequence. This supports the suggestion of a separate Indus sub-plate¹⁶, but for an entirely different reason to that proposed. Originally the proximity of the Salt Range poles to those from the Bhandar and Rewa Series seemed to suggest that they were all of the same age¹⁴. A more likely late-Precambrian age for the Bhandar and Rewa Series now supposes that the structural setting of the Salt Range has caused their poles to be displaced from the Cambrian part of the curve. The pole from the Bolivian sediments of supposed Ordovician age falls in a region which suggests that these sediments may be rather older than supposed.

If the African part of the apparent polar wander path is to be extended back from the late Precambrian group it should continue southwards (Fig. 2) to the vicinity of the Mbozi Complex pole. It is at this point that our interpretation differs significantly from that of Piper *et al.*¹. The early Palaeozoic section of the pole path, as we have drawn it, represents the South Pole path (Fig. 2), but our interpretation obliges us to join up with what Piper *et al.*¹ regard as the North Pole path for about 700 Myr and older (Fig. 4 in ref. 1).

Unity of Gondwanaland

The 745 Myr Malani rhyolite pole for India plots close to a new Australian pole (J. W. Giddings, and A. R. Crawford, unpublished information) for which a provisional Rb–Sr age of 750 Myr has been estimated. These poles fall on the African curve near poles dated at around 800 Myr. This suggests to us that the consistency of the results for the Smith–Hallam reconstruction¹⁶ defines a single pole path extending back at least 750 Myr. It confirms the conclusion that the Pan-African orogenic belts must have been formed *in situ*¹. We can now extend the case of the intercratonic belts within Africa to those within Gondwanaland, such as the Mozambique belt and the eastern Ghats belt.

Late Precambrian glaciations

The occurrence of glacial deposits (tillites) in many late Precambrian formations throughout the world has led to the hypothesis that a widespread glaciation occurred just before Cambrian times^{22,23}. An essential point relating to this hypothesis is whether these glaciations occurred successively throughout polar migration or whether they occurred simultaneously and therefore perhaps extended to low latitudes. The interpretation of the late Precambrian to early Palaeozoic apparent polar wander path for Africa is crucial in this context because many of the late Precambrian glacial deposits are located in that continent. The pole path deduced by Piper¹¹ and Piper *et al.*¹ implies that the glaciated regions essentially occupied low latitudes, and that the glaciation was widespread¹². Our interpretation of the late-Precambrian to Cambrian pole path leads, however, to the conclusion that the pole migrated across South America (which was then joined to Africa) from south to north, during the late Precambrian. This is consistent with the occurrence of glacial deposits which are successively younger northwards along western Africa (Fig. 3).

In South West Africa two glacial episodes are recorded²⁴. Glacial horizons occur in the upper part of both the Damara

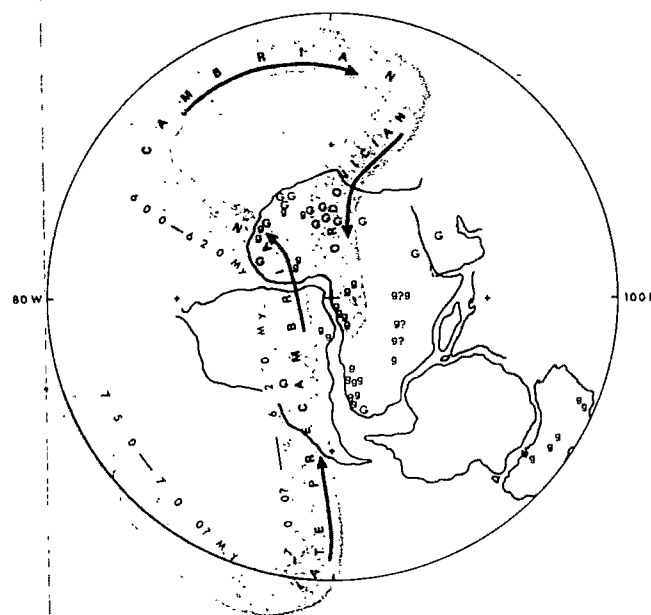


FIG. 3 The revised late Precambrian to early Palaeozoic apparent polar wander path for Gondwanaland compared with the distribution of glacial tillites. G.g. represent Ordovician and late Precambrian occurrences respectively. Glacial deposits in Africa possibly older than about 700 Myr and therefore not related to the drawn path have question marks. Successively younger late Precambrian occurrences along the west coast of Africa are matched by the south–north migration of the pole.

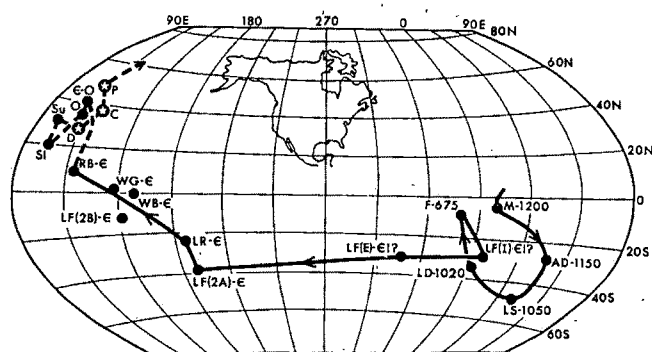


Fig. 4 Suggested revised North Pole apparent polar wander path for North America, which joins the Great Logan Loop³⁵ (1,200–1,020 Myr) to the Ordovician (O) part of the curve by means of a large Cambrian shift. Cambrian poles from: RB, the Ratchliffe–Brook formation¹⁸; WG, Wichita granites¹⁸; WB, Wilberns formation³⁹; LR, Lodore formation¹⁸; LF-I, 2A, 2B and E, Lamotte formation³⁸. Anchor points for the Great Logan Loop³⁵ are shown together with the Franklin pole³⁵ (F). Post-Cambrian Palaeozoic poles³⁹ are joined by a dashed line, the stars representing mean Devonian (D), Carboniferous (C) and Permian (P) poles.

and Nama Systems which are correlated with one another, and whose lower boundaries are not older than 650 Myr. Immediately underlying the Nama System are the Blaubecker Formation glacials and the Buschmannsklippe tillite. These are correlated with glacials of the Numees Formation which forms the upper part of the Gariep Group. A felsite lava in the Kapok Formation, which corresponds to the lower part of the Gariep Group, has an age of 720 Myr. The Blaubecker–Buschmannsklippe–Numees glacials are therefore very likely to be younger than 700 Myr. In Katanga the Petit Conglomerate is probably of glacial origin and has a minimum age of 620 Myr (ref. 25). The Grand Conglomerate of Zaire is, however, probably older than 850 Myr (ref. 25) and therefore relates to a very much earlier glacial episode. In north-western Africa the Saharan 'Eocambrian' tillite has been dated at 620–650 Myr (ref. 26), whereas, in Ghana the Oti tillite has an age of 620 Myr (ref. 29). This succession strongly suggests that the centres of glaciation followed the path of the pole in late Precambrian times as it swept from south to north, past southern, western and north-western Africa, between about 700 Myr ago and 620 Myr ago (Fig. 3). It seems significant that the best dated African late-Precambrian pole, the 630 ± 24 Myr Ntonya result, plots just off north-west Africa (Fig. 2) corresponding to the time when this region was extensively glaciated.

During the Cambrian the broad trends of climatic change are perceptible²⁸. In North Africa the occurrence of thick successions of Lower Cambrian warm water deposits include also archaeocyathid reefs. Cambrian rocks are frequently brightly coloured, indicating a warm climate, but they give way to more drab Ordovician sequences which culminate in the great, late Ordovician, Saharan glaciation²⁹. These broad trends are not only compatible with the Cambrian and Ordovician sections of the pole path, but also resolve a previously puzzling palaeoclimatic anomaly³⁰. From near polar conditions in north-west Africa the broad loop taken by the pole during the Cambrian placed North Africa in low latitudes, with a return to intermediate, and finally polar, latitudes during the Ordovician.

It is not clear how the pattern of late Precambrian glaciation in Australia fits into the polar migration proposed in Figs 2 and 3. Dunn *et al.*³¹ cite ages of 750 Myr for the Sturtian–Moonlight Valley glaciation, but these are all based upon Rb–Sr measurements on shales. The interpretation of isochrons drawn through such data in terms of age of deposition is very much an open question (W. Compston, personal communication), so that the relationship of these ages to

those on our pole path is unclear. We make the general observation, however, that between 750 Myr and 600 Myr a polar migration of at least 180° is proposed with respect to Gondwanaland. Very large polar shifts are observed in almost all continents during the late-Precambrian and Cambrian. It is therefore not surprising that the poles migrated quite rapidly over different parts of the globe leaving records of glaciated regions as they passed over the various continents. In these circumstances it is not necessary to invoke the hypothesis of an extensive synchronous world-wide glaciation that extended to low latitudes (see also ref. 32). The decisive test between the two hypotheses will come from palaeomagnetic measurements on the actual glacial horizons in various places around the world. If these measurements show that many of these deposits were formed in low latitudes then this will support the hypothesis of a synchronous world-wide glaciation.

Great Logan Loop of North America

There is an impressive correspondence between an extended loop in the African apparent polar wander path and the so-called Great Logan Loop³³ of the North American path^{1,11}. Both of these loops cover the time range 1,200–1,000 Myr. Our interpretation of the African polar wander path means that the poles in this time range are now south poles which can only be matched with the presumed North American north poles for the same time. The fit of the two loops is impressive, and so we tentatively suggest that the late Precambrian to early Palaeozoic section of the North American polar wander path may be much more complicated than previously supposed. Irving and Park³⁴ have already noted the very tentative nature of this section of the North American curve. There is only a single pole (the Franklin pole, 675 Myr) between the end of the Logan loop at about 1,000 Myr, and the Cambrian. During the same period in Africa the polar shift was more than 180° (Fig. 2). During the Cambrian alone the pole shift was about 90° . Furthermore, Cambrian results from the Bohemian massif have previously been interpreted in terms of a large polar shift of about 130° (ref. 35) and a large polar shift is also recorded in the Cambrian of Siberia³⁷.

The Lamotte formation of Missouri (Cambrian in age, although the fossil evidence is poor) shows four distinct palaeomagnetic poles³⁸. Poles from the higher horizons are in two groups (2A and 2B), related to their stratigraphical positions, and suggest a polar migration during the Late(?) Cambrian. Poles from the lower horizons seem to be anomalous. No reason is apparent for group 1 and the other (Locality E) is close to the Carboniferous pole for North America. Carboniferous remagnetisation has been proposed, although no evidence has been given either to suggest how this arose or why it occurred selectively at only one locality. Studies of the Ordovician Trenton Limestone³⁷ have shown that the hypothesis of widespread late-Palaeozoic remagnetisation of Lower Palaeozoic rocks is no longer tenable. We therefore interpret these poles as representing a polar migration during the Cambrian.

Figure 4 shows how it is possible to join the later Palaeozoic poles for North America through an extended Cambrian path, to the opposite poles of the Logan Loop. These poles become the north pole path for North America with the consequence that the plot of the Logan Loop in the Pacific^{33,34} represents the south pole path. It is then readily matched with the African loop (see ref. 1). Our prediction therefore is that extensive studies of late Precambrian and Cambrian rocks of North America (1,000–500 Myr) will reveal a complicated apparent polar wander path with a polar shift through at least 180° .

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Chimaera mouse study shows absence of disease in genetically dystrophic muscle

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Chimaeras have been derived from aggregation of normal and dystrophic mouse embryos which had healthy muscles of almost totally dystrophic genotype, and diseased muscles of normal genotype. These results suggest that extramuscular factors must be implicated in muscular dystrophy.

UNTIL recently muscular dystrophy in man and animals was considered to result from a genetically determined primary defect within the muscle fibres themselves. Evidence is now accumulating which suggests that dystrophic individuals have an abnormality within the nervous system which may itself be largely responsible for the muscular disorder; namely, an abnormal trophic influence of the motor neurones on the affected muscles^{1,2}.

One ideal situation which could allow the investigation of the site of the primary genetic lesion would be an adult animal in which either genetically dystrophic muscle was innervated by a normal nervous system or genetically normal muscle was innervated by a dystrophic nervous system. The mouse chimaera offers an opportunity to approach this ideal situation without requiring that the animal undergo surgical manipulations, with subsequent establishment of regenerative processes³.

Mouse chimaeras are derived from the aggregation of pre-implantation embryos (each of eight cells) of differing

genotypes. These mosaic morula are cultured for 24 h (in which time they differentiate to single blastocysts) and are then transferred to the uterus of pseudopregnant females to complete development⁴. The chimaeras of interest here would derive from aggregation of dystrophic and normal embryo types. It was hoped that the resulting *in vivo* situation would make possible the study of the interaction between genetically defined muscle and the rest of the organism during development, and in the mature state.

In these chimaeras, if muscles of identified dystrophic genotype were found to have normal characteristics it would be unlikely that the disease could be due to a primary myopathy. In addition, if genetically normal muscle showed dystrophic characteristics, then this too would support the inference that there was an extramuscular component of the disease. Conversely, a direct correlation between dystrophic genotype and dystrophic characteristics would support the original myogenic hypothesis.

The principal requirement was to have an adequate genetic marker with which to determine the muscle genotype. Amongst the inbred strains of mice there are several suitable markers, and for this experiment the electrophoretic isozymes of malic enzyme⁵ were incorporated as the genotype marker, for the mature muscles of the dystrophic-normal chimaeras.

An important feature of the proposed *in vivo* preparation is that the muscle would be investigated at a time when, in the lifespan of an animal with the dystrophic genotype, the

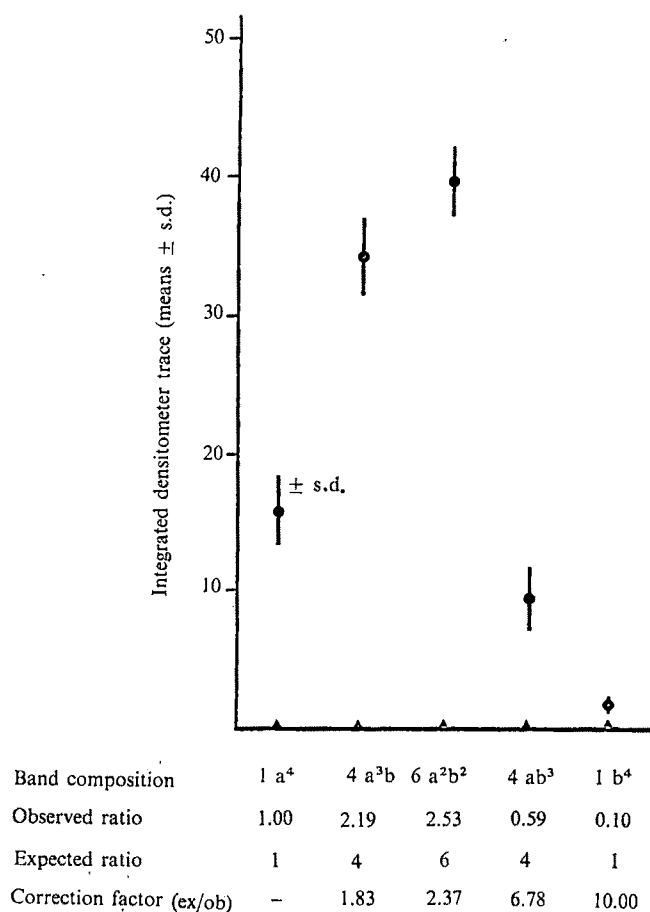


FIG. 1 Activity ratio of *Mod-I* isozymes in the heterozygote pattern from muscle supernatant. Correction factors were applied to densitometer results of chimaera muscle before calculation of relative *Mod-I^a/Mod-I^b* nuclei.

disease would be clearly shown⁶. The slow clinical progression of the disease in dystrophic mice with the new *dy²³* allele allows these mice to mate⁶, and homozygous dystrophic morula were obtained by direct mating of dystrophic pairs. For the experiment seven chimaeras of dystrophic \longleftrightarrow normal (C57Bl/6J *dy²³/dy²³* \longleftrightarrow SWV) and two of normal \longleftrightarrow normal (C57Bl \longleftrightarrow SWV) composition, all of which demonstrated coat colour and haemoglobin mosaicism, were produced. These were examined daily (birth to 2 months) and weekly for the following 4 to 10 months in different animals, for clinical features of muscular dystrophy.

An estimate of gross muscle function in the mature chimaeras was obtained by recording the maximum isometric twitch of their anterior tibialis muscles in response to supra-maximal nerve stimulation.

The histological appearances of the muscles from chimaeras were compared with those of normal and of dystrophic mice. Individual identified muscles were dissected out and a 2 mm section, taken across the middle of the muscles, was processed for subsequent preparation of 1 μ m Epon sections; the remainder of the muscle was frozen in liquid nitrogen and stored at -70° C for subsequent genotype analysis.

Genetic marker

The dystrophic embryos used in this chimaera preparation had the *Mod-I^b* genotype for malic enzyme (E. S. Russell, personal communication). The normal embryos were determined to have a *Mod-I^a* genotype for the same enzyme. The subsequent analysis depended on the recognition and relative quantitation of the *Mod-I^a* and *Mod-I^b* gene products in the muscles investigated. This was achieved by starch gel electro-

phoresis, which enables the clear resolution of the isozymes of malic enzyme. *Mod-I^a* and *Mod-I^b* genes code for the subunits of the enzymatically active tetramere, malic enzyme⁷. In the *Mod-I^a/Mod-I^b* heterozygote where a and b subunits are simultaneously produced⁸ and randomly associate, five tetrameric forms, aaaa, aaab aabb, abbb, bbbb are electrophoretically resolved. In the dystrophic \longleftrightarrow normal chimaeras, the presence of b subunits observed by resolution of the *Mod-I^b* isozyme or hybrid bands indicates the contribution of genetically dystrophic nuclei. Subunits of a type, forming the *Mod-I^a* isozyme or hybrid bands, could only be derived from the genetically normal nucleus. The descriptions of nuclei as 'normal' or 'dystrophic' is based upon this isozyme characterisation. The hybrid band feature of this isozyme system was used to differentiate muscles with mosaic fibres, that is, fibres with both normal and dystrophic nuclei within the same cytoplasm, from muscles where there were mixtures of fibres, each of which had only normal or dystrophic nuclei, but not both. In the latter case, only the parental bands, aaaa and/or bbbb would be detected⁹.

If the enzymatic activities of the five tetrameric types were equal, in the heterozygote, the theoretically expected 1:4:6:4:1 ratio from random association of the subunit types into tetrameres would be observed in the ratio of activity developed on the starch gel. This ratio was not observed in the original description of the *Mod-I* tetrameric types⁷ nor was it seen in the electrophoretic system used in the present experiment. The measured density ratio of the five bands in the heterozygote after enzymatic staining on the starch gel was 1.00 aaaa, 2.19 aaab, 2.53 aabb, 0.59 abbb, and 0.10 bbbb (Fig. 1). The assumption was made therefore that each tetramere type had a unique enzymatic activity. To measure the *Mod-I^a* and *Mod-I^b* gene products the observed activity for each tetrameric type was corrected by unique factors to regenerate the theoretical 1:4:6:4:1 ratio. These factors, derived from heterozygote electrophoretograms, were subsequently applied to the electrophoretograms of the chimaera muscles before calculation of the relative *Mod-I^a* and *Mod-I^b* genotypes.

Muscle analysis

The behaviour of all the chimaeras was not distinguishable from that of normal animals of the same age. At no time could convincing features of muscular dystrophy be demonstrated by any of the recognised stresses and manipulations known⁶.

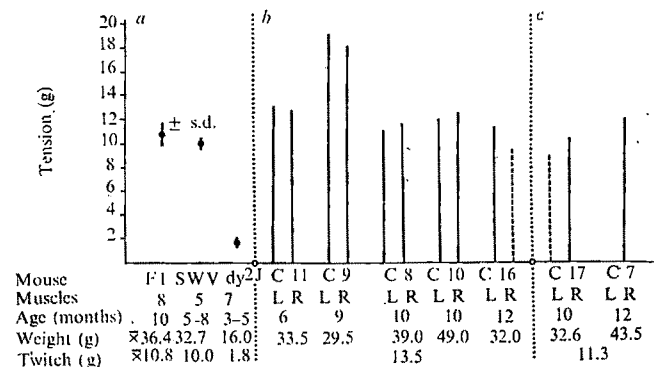


FIG. 2 Maximum isometric twitch of anterior tibialis muscles. Mouse: F1 is SWV-C57Bl/10J; *dy²³* is C57Bl/6J *dy²³/dy²³* dystrophic; C is chimaera (C11, 9, 8, 10, 16 are dystrophic \longleftrightarrow normal; C17 and 7 are normal \longleftrightarrow normal). Muscles: numbers are number of muscles analysed; L and R is left and right; broken lines represent suboptimal preparations and are not included in means; C7 left was a technical failure. a, control; b, dystrophic chimaeras; c, normal chimaeras.

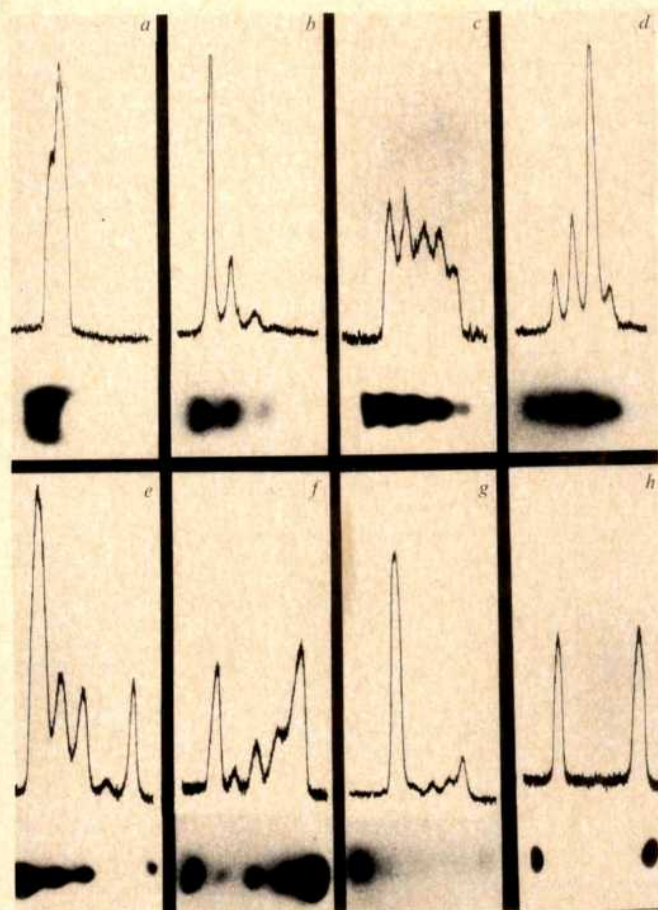


FIG. 3 *Mod-I* electrophoretograms and corresponding densitometer traces from C57Bl/6J $dy^{23}/dy^{23} \longleftrightarrow$ SWV $^{+/+}$ chimaera muscles. *Mod-I^a* isoform is on the left of each trace. Frozen muscle samples were weighed, homogenised in 1 ml of H₂O; centrifuged at 3° C for 15 min at 15,000g; the supernatant at 90,000g for 30 min, and freeze dried. The supernatant was reconstituted with H₂O to yield a seven-fold concentration before electrophoresis. Electrophoresis was carried out on a microstarch gel system¹². The buffer system was modified for high voltage (21 V cm⁻¹ after Baker and Mintz⁹). The bridge consisted of 2,000 ml H₂O, 18.8 g citric acid (anhydrous) and Tris base to pH 8.4. Gel buffer was 12.5 ml of bridge diluted to 250 ml with H₂O and re-adjusted to pH 8.4 with Tris base. Electrophoresis was performed at 5° C for 5 h. Malic enzyme was demonstrated by the assay of Henderson¹³. Densitometry was carried out directly with glycerine-cleared gels on a Joyce Loebl recording microdensitometer. The trace was integrated by planimetry and the relative a and b subunit composition was calculated.

All the anterior tibialis muscles investigated showed twitch tensions which were either equal to or even larger than those of normal animals which were examined in parallel (Fig. 2).

Electrophoretograms from the majority of dystrophic \longleftrightarrow normal chimaera muscles revealed the activity of both dystrophic and normal nuclei (Fig. 3). The constitutional genotype of the muscles examined ranged from completely normal (Fig. 3a) through many heterozygous-like forms (Fig. 3b-g) to muscles with a mixed population of totally normal and totally dystrophic fibres (Fig. 3h). Although no muscles without some normal nuclei were observed, in some muscles as many as 94% of the nuclei were estimated to be of dystrophic genotype. Amongst these muscles with a high proportion of dystrophic nuclei, some had very large populations of fibres with only dystrophic nuclei (Fig. 3h). The dystrophic nuclei composition of 47 muscles, pooled from five chimaeras, was 58%. This estimate of dystrophic geno-

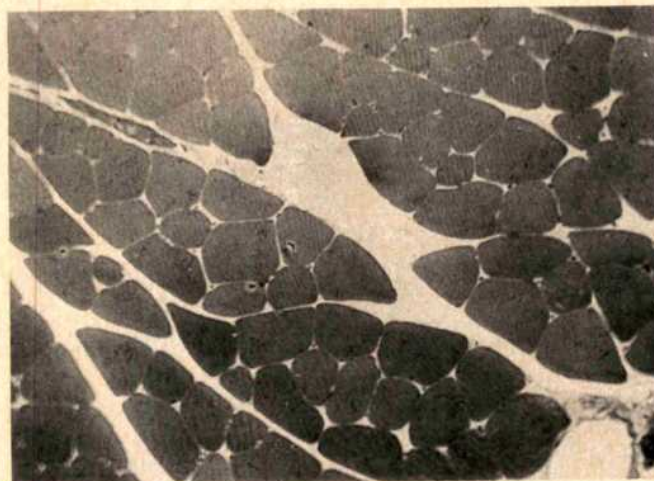


FIG. 4 Left biceps femoris muscle of chimaera No. 10. Note the normal morphology of muscle fibres. Magnification $\times 153$ approximately.

type indicates that no obvious selective death of genetically dystrophic nuclei had taken place either during myogenesis or in the mature muscle.

In the two normal \longleftrightarrow normal chimaeras, in which the *Mod-I^b* subunits were derived from C57Bl nuclei without the dystrophic genotype, 10 muscles were pooled in a similar fashion for genotype analysis. In these, the *Mod-I^b* genotype was estimated to be 82%. This higher value for *Mod-I^b* nuclei probably reflects the small sample size, and not a selective advantage of *Mod-I^b* cells, since individual muscles of these normal chimaeras had from 3% to 94% *Mod-I^b* nuclei.

Since the hind limbs show the earliest and most extensive dystrophic clinical symptoms in dystrophic mice, histological examination was made of hind limb muscles in chimaeras which demonstrated extremes of genetic constitution. Minor features of dystrophic muscle pathology, such as central nucleation, were observed in very few fibres in every dystrophic \longleftrightarrow normal chimaera muscle examined. No correlation of these minimal pathological features and the constitutional genotype was observed. Thus in Fig. 4, the left biceps femoris muscle of chimaera No. 10, had 83% dystrophic genotype, and the only detectable abnormality was the occasional central nucleus. Conversely, the left biceps femoris of chimaera No. 16, with no detectable dystrophic

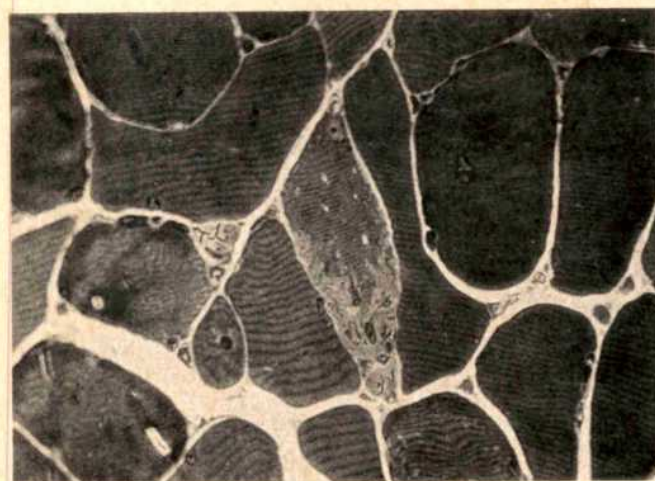


FIG. 5 Left biceps femoris muscle of chimaera No. 16. Note frequency of central nucleation and the presence of coagulation necrosis. Magnification $\times 510$ approximately.

genotype, demonstrated relatively more extensive pathological involvement than all the other muscles examined, that is, degenerating fibres and fibres with central nucleation grouped across the sections (Fig. 5).

These results from chimaeric mice are not compatible with a primary myopathy in murine muscular dystrophy. It could be argued that a small population of normal nuclei, which support synthesis of some postulated chemical, deficient within the fibres of dystrophic muscle, could prevent dystrophic pathology. This seems unlikely, however, because muscles with a high or very high dystrophic genotype, including those with large populations of completely dystrophic fibres, appeared otherwise normal. Furthermore, genetically normal muscle showed pathological changes; which suggests that, at least in the mosaic environment, some entirely extramuscular factor is primarily responsible for the muscle degeneration.

Finally, it has been suggested that these artificial chimaeras show mosaicism similar to that expressed in human females as a consequence of random inactivation of one X chromosome¹⁰. A variable muscle constitutional genotype has long been considered to account for the variable pathological features observed in females carrying the X-linked form of Duchenne muscular dystrophy¹¹. If the primary lesion in Duchenne dystrophy is homologous to that in murine dystrophy, this concept must be re-evaluated. A variable mosaicism in some other cell type, possibly the motor neurones, could account for this observed clinical variability.

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Gene dosage compensation in metafemales (3X; 2A) of *Drosophila*

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The X chromosome of Drosophila provides a unique system for the study of modulation of eukaryotic gene activity.

IN *Drosophila*, dosage compensation, that is, the equalisation of X-linked gene products in males and females, has been reported for RNA synthesis, enzyme activities and terminal phenotypic products¹⁻³. In contrast to mammals, where dosage compensation seems to be mediated by inactivation of one of the two X chromosomes in somatic cells of diploid females, there is good evidence that in *Drosophila* both X chromosomes are active in all cells of the female soma⁴ although the level of activity of each is half that of the single X in a male.

This observation has been extended recently to triploid females (3X;3A where A is one set of autosomes)⁵⁻⁸. Furthermore, in male-like triploid intersexes (2X;3A), the X chromosome was reported to function at a level intermediate between the normal male and female levels. Thus X-linked gene activity seems to be correlated with the rela-

tive number of X chromosomes and sets of autosomes in the genome: activity is high in males where the X/A ratio is 0.5, intermediate in triploid intersexes where the X/A ratio is 0.66 and low in diploid or triploid females where the X/A ratio is 1.0. To test this correlation we have measured X-linked and autosomal gene activity in metafemales (3X;2A) where the higher X/A ratio of 1.5 might be expected to yield a relative activity per gene dose even lower than that characteristic of females. That this may be the case was suggested by the observation that metafemales and females homozygous for an eye colour hypomorphic mutation have the same phenotype⁹. Our results indicate that enzyme activity and rates of RNA synthesis are similar in metafemales and in normal females and, therefore, that X-linked gene activity expressed on a per gene basis is less in these metafemales than in diploid or triploid females.

We used laboratory stocks of *D. melanogaster* and the genetic markers and symbols are those described by Lindsley and Grell¹⁰. Various doses of the structural gene for 6-phosphogluconate dehydrogenase¹¹ (*Pgd*⁺) were obtained using *T*(1 → 3)*w*^{sc}. This rearrangement consists of a transposition of a small segment of the X chromosome, including *Pgd*⁺, into chromosome 3. The two elements of the

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TABLE 1 6PGD activity for various structural gene doses in male and female larvae

Sex	Experiment†	No. of <i>Pgd</i> ⁺	6PGD	Enzyme activities*		
				G6PD‡	ADH	IDH
Males	A	1	6.3 ± 0.2	7.1 ± 0.1	35.9 ± 0.5	14.6 ± 0.3
		2	13.0 ± 0.6	8.4 ± 0.1	38.8 ± 0.9	17.8 ± 0.3
	B	1	6.0 ± 0.4	7.4 ± 0.3	31.0 ± 1.8	14.9 ± 0.7
		2	11.5 ± 0.5	8.3 ± 0.0	35.3 ± 1.1	17.7 ± 0.8
Females	A	2	6.4 ± 0.4	6.9 ± 0.2	35.6 ± 1.0	16.2 ± 0.4
		1	3.5 ± 0.2	6.2 ± 0.3	33.8 ± 2.5	17.4 ± 0.5
	B	2	5.9 ± 0.5	6.4 ± 0.2	38.1 ± 4.3	13.6 ± 0.8
		3	9.2 ± 0.3	7.6 ± 0.1	39.3 ± 1.6	17.2 ± 0.4

* Expressed as mean μmol coenzyme reduced $\times 10^2$ per min per mg protein \pm s.e. (No. of determinations per mean = 4.)

† Experiment A, flies produced by mating *e/e* females to *Df(1)w^{sc}, v f/Y*; *Dp(1 → 3)w^{sc}, e⁺/e* males. Experiment B, flies produced by mating *e/e* females to *y w spl/Y*; *Dp(1 → 3)w^{sc}, e⁺/e* males.

‡ These values are corrected for an estimated 6% level of 6PGD activity in the G6PD assay using an extract from normal females.

translocation are *Df(1)w^{sc}*, the deficient X chromosome, and *Dp(1 → 3)w^{sc}*, the duplication-bearing autosome. Females homozygous for the recessive marker *ebony* (*e*) were crossed to either *Df(1)w^{sc}, v f/Y*; *Dp(1 → 3)w^{sc}, e⁺/e* males or to *y w spl/Y*; *Dp(1 → 3)w^{sc}, e⁺/e* males. Full-grown third instar larvae were collected and sorted according to sex and *ebony* (black spiracles) or non-*ebony* (wild type, brownish spiracles) phenotype. The first cross yields *ebony* male and female larvae bearing one dose of *Pgd*⁺ and non-*ebony* male and female larvae bearing two *Pgd*⁺ doses. The second cross yields *ebony* male larvae bearing one *Pgd*⁺ dose, non-*ebony* male and *ebony* female larvae bearing two *Pgd*⁺ doses, and non-*ebony* female larvae possessing three doses of *Pgd*⁺.

To produce metafemale larvae, *C(1)RM, y v f/Y* attached-X females were crossed to *y v f-y⁺/Y* males. (The X chromosome represents an X-ray-induced detachment of the attached-X, marked with a small duplication bearing the wild type, dominant allele of *yellow*, *y⁺*.) Female larvae with yellow mouth parts and setae were controls (2X;2A), those with black mouth parts and setae were metafemales, while all male larvae had black mouth parts. To improve the yield of emerged adult metafemales, *C(1)RM, y v f/Y* females were outcrossed to *+ / Y* males of a Samarkand wild-type line. The phenotypic distinction of the various larval types was the same as in the previous cross. While crosses were usually incubated at $25^\circ \pm 1^\circ \text{C}$, some cultures of attached-X females by wild type males were maintained at $20^\circ \pm 1^\circ \text{C}$ to obtain larvae for autoradiography.

Larval samples were homogenised in conical ground-glass

homogenisers at a ratio of fifteen larvae per ml of distilled water; adult samples were homogenised at a concentration of 20 mg live weight per ml of distilled water. After 20 min, samples were centrifuged for 40 min at 12,000 *g* and the clear supernatant was used as a crude enzyme extract. Throughout preparation all extracts were maintained at 0° – 5°C .

Larval fat body samples were obtained by chopping 50 mature third instar larvae with a razor blade in a few drops of 2% sucrose. After transfer to a test tube containing approximately 5 ml of cold 2% sucrose, fat bodies and other organ fragments were dislodged from the body wall casings by aspirations into a Pasteur pipette. After standing for 20 min at 0°C , most of the loose fat body fragments floated to the surface, remarkably free of other tissue. The fat body layer was drawn off and its volume adjusted to 1 ml with distilled water. After brief sonication, the extract was centrifuged for 20 min at 12,000 *g* and enzyme activities were determined in the supernatant.

We measured activities of glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), α -glycerophosphate dehydrogenase (α -GPDH, EC 1.1.1.8), NADP-dependent isocitrate dehydrogenase (IDH, EC 1.1.1.42), and alcohol dehydrogenase (ADH, EC 1.1.1.1). Substrate solutions and assay conditions for the first four enzymes have been described before⁵. ADH was measured using the reaction mixture of Jacobson *et al.*¹². All dehydrogenase activities were monitored spectrophotometrically at 340 nm and enzyme activities are expressed as μmol of NAD⁺ or NADP⁺ reduced

TABLE 2 Enzyme activities in metafemales and euploid controls

Material	Sex	6PGD	Enzyme activities*		
			G6PD	α -GPDH	IDH
Whole larvae	Males	6.6 ± 0.2† (5)	8.0 ± 0.6 (7)	16.3 ± 1.1† (7)	21.3 ± 0.3 (7)
	Females	7.3 ± 0.2† (5)	7.4 ± 0.4 (10)	16.2 ± 0.8† (10)	22.3 ± 0.6 (10)
	Metafemales	8.5 ± 0.3 (4)	8.0 ± 0.3 (9)	22.3 ± 1.1 (9)	23.8 ± 0.6 (9)
Fat body	Males	9.4 ± 0.4† (4)	7.0 ± 0.3† (4)	14.3 ± 1.0† (4)	11.9 ± 0.6† (4)
	Females	9.1 ± 0.7† (4)	7.6 ± 0.5† (4)	14.6 ± 0.8† (4)	11.6 ± 0.4† (4)
	Metafemales	14.4 ± 0.5 (5)	10.8 ± 0.2 (5)	23.4 ± 0.7 (5)	13.9 ± 0.4 (5)
0–1 h adults	Males	7.4 ± 0.2 (6)	6.4 ± 0.2 (6)	63.9 ± 2.4† (6)	29.4 ± 0.7† (6)
	Females	7.6 ± 0.2 (6)	8.4 ± 0.2 (6)	59.9 ± 2.0† (6)	29.3 ± 0.3† (6)
	Metafemales	7.4 ± 0.4 (5)	7.0 ± 0.7 (5)	44.6 ± 1.2 (5)	24.4 ± 0.8 (5)
9–11 h adults	Males	10.1 ± 0.6 (2)	8.3 ± 0.4 (2)	97.7 ± 3.6 (2)	35.8 ± 1.0 (2)
	Females	8.6 ± 0.5 (2)	9.4 ± 0.4 (2)	86.4 ± 3.2 (2)	33.4 ± 1.6 (2)
	Metafemales	9.2 ± 0.1 (2)	10.1 ± 0.1 (2)	77.2 ± 1.2 (2)	34.6 ± 0.0 (2)

* Expressed as mean μmol coenzyme reduced $\times 10^2$ per min per mg protein \pm s.e. Number of determinations are indicated in parentheses.

† Male or female values differ from metafemale values at the 0.05 level of significance.

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TABLE 3 Normalised X-linked enzyme activities per gene dose in metafemales and females

Material	Sex	6PGD/ α -GPDH	6PGD/IDH	G6PD/ α -GPDH	G6PD/IDH
Whole larvae	Females	0.21 \pm 0.03	0.17 \pm 0.02	0.23 \pm 0.02	0.17 \pm 0.02
	Metafemales	0.11 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01	0.11 \pm 0.01
Fat body	Females	0.31 \pm 0.04	0.39 \pm 0.03	0.26 \pm 0.02	0.34 \pm 0.05
	Metafemales	0.21 \pm 0.01	0.37 \pm 0.03	0.16 \pm 0.02	0.26 \pm 0.03
0-1 h adults	Females	0.063 \pm 0.003	0.13 \pm 0.01	0.071 \pm 0.003	0.14 \pm 0.01
	Metafemales	0.055 \pm 0.010	0.10 \pm 0.01	0.052 \pm 0.014	0.09 \pm 0.02
9-11 h adults	Females	0.051 \pm 0.002	0.13 \pm 0.01	0.054 \pm 0.000	0.14 \pm 0.01
	Metafemales	0.040 \pm 0.004	0.09 \pm 0.003	0.044 \pm 0.002	0.10 \pm 0.00

Activities are expressed as mean standardised units \pm s.e.

per min per mg protein at 29° C. Extract protein concentration was determined using the method of Lowry *et al.*¹³. Mean enzyme activity values were compared using Student's *t* test.

For autoradiography, salivary glands were dissected out of late third instar larvae in Ephrussi-Beadle solution. They were incubated for 5 min in ³H-uridine (Amersham Searle, 30 Ci mmol⁻¹) at a concentration of 20 μ Ci ml⁻¹. Fixation and squashing, preparation, exposure and development of autoradiographs were as previously described⁷. The rate of RNA synthesis was measured in segment 1-3C of the X chromosome using an autosomal segment—the right arm of chromosome 2 (58F-60)—as an internal control. Grains were counted in nuclei where both segments were unobstructed. Average values of grain counts over the X segment (\bar{X}) and the autosomal segment ($\bar{2R}$) were obtained for every gland. The slope of the regression line of \bar{X} on $\bar{2R}$ is a measure of the average increment in the number of grains over the X which accompanies a unitary increment in the number of grains over the autosome. This slope, represented by the regression coefficient *b*, is a measure of the rate of RNA synthesis in the X relative to that in an autosome.

Enzyme levels

Before examining the dosage compensation of metafemale enzyme levels in larvae, we had to establish that the relationships between regular adult male and female enzyme levels are also evident in larvae. As Table 1 shows, we found this to be the case. Two of the four enzymes studied, 6PGD and G6PD, are X-linked^{11,14} while the known structural genes for ADH and IDH are linked to chromosomes two and three, respectively^{15,16}. The autosomal enzyme levels of male and female larvae are equivalent and dosage compensation seems to be fully operative, as evidenced by the similarity in G6PD and 6PGD activities in the two sexes, and by the observation that each *Pgd*⁺ dose in female larvae with one, two or three doses of this gene produces approximately half the amount of 6PGD that it produces in male larvae with one or two doses of the gene.

Enzyme activities of metafemales and normal diploid females are presented in Table 2. Activities of male sibs are

included as an internal control. Measurements were performed on extracts of whole larvae, larval fat body, 0-1 h and 9-10-h-old emerged adults. α -GPDH, the gene for which is located on the second chromosome¹⁷, was measured instead of ADH in these experiments. No male and female values differed from each other. Metafemales, on the other hand, showed greater variability, with values often in excess of those of control females. In most cases, however, both X-linked and autosomal enzymes seemed to be affected. For example, metafemale larvae have significantly higher specific activities of 6PGD and α -GPDH than euploid controls. This may be the consequence of the longer time required by metafemales to reach the end of the third larval instar, leading to the preferential accumulation of certain soluble proteins. When we measured enzyme activities in the larval fat body, which is metabolically highly active, all soluble dehydrogenases assayed were significantly higher in metafemales than in euploid controls. Emerged adult metafemales, on the other hand, had activities either reduced (α -GPDH and IDH in 0-1 h-old adults) or equivalent to those of controls.

X-linked enzyme activity measurements can be normalised in relation to either α -GPDH or IDH activity, since metafemales and control females each contain two doses of the structural genes of these enzymes per genome. Table 3 contains per gene activities obtained by dividing the normalised X-linked activities of metafemales by three and of females by two. Taken together and evaluated in the light of the generalised physiological disturbances of metafemales, these results indicate that X-linked genes are substantially less active in metafemales than in normal females, and that the presence of a third X chromosome in the genome of the former flies does not mediate a structural gene dosage response, that is, a 50% increase in X-linked gene product per cell. This conclusion is substantiated by the autoradiographic monitoring of X chromosome activity.

RNA synthesis

The results of the study of ³H-uridine incorporation by metafemale and diploid female larval salivary gland chromosomes are presented in Table 4. To compare metafemale and female grain counts properly, it must be remembered that in the former the X element contains three synapsed chromo-

TABLE 4 RNA synthesis in metafemales and females

Sex	No. nuclei	No. glands	Average [X]	Average [2R]	<i>r</i>	<i>b</i> \pm s.e.
Metafemales	76	9	59.17	44.74	0.98	0.96 \pm 0.07
Females	84	9	60.52	52.13	0.97	1.10 \pm 0.10

Average [X] and average [2R] = overall average of grain counts over X and 2R segments, respectively.

r, Correlation coefficient of the mean grain counts for individual glands, [X] and [2R].

b, Regression coefficient of [X] on [2R]. Not significantly different.

The metafemale values for average [X] and *b* were corrected for differential self-absorption.

somes instead of two and therefore absorbs more β particles than does the X element of control females. Taking the efficiency of X chromosome grain production in males as approximately 20% higher than in females⁷, the values for meta-females were corrected by an increment of 10%.

If the grain counts over the X and autosome are averaged for each gland and a regression line of these averages ($[X]$ on $[2R]$) is established, the slope of this line is the same in males and females (G. Maroni, R. Kaplan and W. Plaut, in preparation) or in triploid intersexes and triploid females⁷. This indicates that the rate of RNA synthesis by the X element relative to the rate of synthesis by the autosomal element is the same in males where the X is single as in females where the X element is made up of two paired X chromosomes; similar conclusions were reached for triploid intersexes (two Xs) and triploid females (three Xs). We

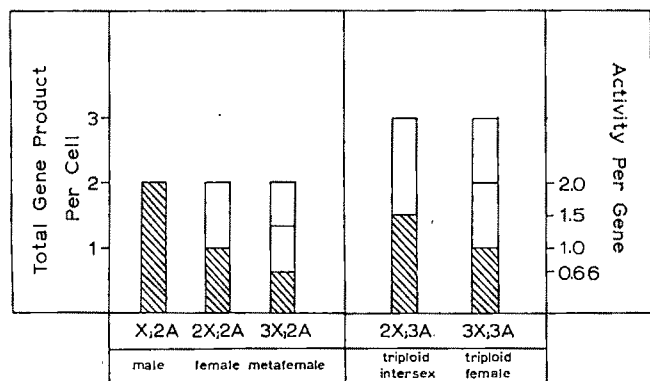


FIG. 1 Schematic representation of X gene activity in individuals of various chromosomal constitutions. The height of the columns represents total gene product per cell; a value of 2 was arbitrarily chosen for diploids. The shaded areas represent activity per gene dose; diploid females were given a value of 1.

found that there was no significant difference between the regression coefficients of metafemales and females, indicating that the rate of RNA synthesis by the trivalent X element of metafemales relative to the rate of RNA synthesis by an autosomal element is indistinguishable from that of normal females. Another way of expressing this result would be to say that an X chromosome in a metafemale synthesises, per unit time, only two-thirds of the RNA which it normally does in a female.

Compensation and modulation

When our findings are pooled with those derived from enzymological and autoradiographic comparisons of diploid males and females^{1,2}, it is evident that the overall expression of X-linked genetic activity (that is, the total amount of X-linked gene product) in diploid cells is independent of the number of X chromosomes within the cell. Similarly, comparisons of triploid genotypes³⁻⁸ suggest that within triploid cells, the phenotypic level achieved by X-linked genes is independent of the number of X chromosomes present in the cell. In triploid cells, however, the overall level of X-linked gene activity is approximately 50% greater than that of diploid cells^{5,8}. Because of the latter relationship, Maroni and Plaut^{7,8} proposed that dosage compensation is mediated by an autosomally-derived factor which is rate-limiting in the expression of X-linked genes. Our current finding with respect to the equivalence of X-linked activity

in metafemales and diploid females agrees with this hypothesis.

In spite of differences in the means of implementation of dosage compensation, there is a similarity between *Drosophila* and mammals. Lyon¹⁸ has suggested that the somatic differentiation of X chromosomes into active or inactive states involves interplay of genes on the X and an autosome. This suggestion is based on the observation that in human aneuploids with two sets of autosomes and varying numbers of sex chromosomes (XXY; 2A,3X; 2A or 4X;2A) only one X remains active, while in triploids (XXY;3A or 3X;3A) or tetraploids (XXYY;4A) two X chromosomes can remain active^{19,20}.

The modulation of gene activity is a central problem in the study of genomic expression. Different levels of induction can be achieved easily with microbial systems by manipulation of effector or terminal product concentrations. Analogous circumstances are much more difficult to establish in the case of multicellular organisms. Therefore, the X chromosome of *Drosophila* is of unique interest in this respect since four levels of activity have been recorded for genes on this chromosome: the highest level is in males, the lowest in metafemales, and two intermediate levels are in triploid intersexes and euploid females (Fig. 1). These relative levels of activity seem to be established comparatively early in development, before the late larval stages. Elucidation of the causative factors responsible for a given level of X-linked gene activity in *Drosophila* should contribute to the understanding of other examples of eukaryotic genome modulation.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Statistical analysis of close pairs of QSOs

THE observation of close pairs of QSOs with very different redshifts has been suggested to be evidence in support of the noncosmological redshift hypothesis^{1,2}. The statistical arguments used by Stockton¹ and by Wampler *et al.*² have recently been attacked by Bahcall and Woltjer³ on the ground that the method used is one of *a posteriori* statistics. Statistics aside, they have ignored the point that in one case², the evidence is not only based on close proximity of the two QSOs but also on two coincidences in wavelengths in the spectra of the two objects. Although the argument against *a posteriori* statistics is correct in the absolute sense, this method is widely used in astronomy and has generally been accepted, except in cases in which the hypothesis under consideration has not reached the point of general acceptability. The redshift controversy is a good example of this. Evidence as to the existence of non-cosmological redshifts must, because we have few ways of measuring distance, depend upon finding conflicts between distances derived from the redshifts (assumed cosmological) and distances derived in other ways⁴—either through statistical arguments or by finding luminous connections between pairs of objects. If, as seems to be the case, most workers use the redshift as the prime criterion of distance, and subordinate other methods of measurement to it, no solution to the problem is possible.

One or two examples of the prejudicial approach used in this problem will suffice:

(1) Several cases of QSOs with low redshift close to small groups of galaxies, and with redshifts similar to those of one or more members of the respective groups, have been found. Although some of these associations may be real, the statistical evidence is not convincing⁵; nevertheless, these juxtapositions are cited by many as proof of the cosmological origin of QSO redshifts. It is instructive to examine some of the analyses which attached statistical significance to such associations. For example, Gunn⁶ found a small group of galaxies near the QSO PKS2251+11 ($z = 0.33$) agreeing in redshift within the observational uncertainty ($\Delta z \approx \pm 0.01$). He finds that the brightest member of the group, with a calculated absolute magnitude -21.2 , lies $28''$ from the QSO; then he asks: "What is the probability of finding a galaxy of absolute magnitude -21 or brighter within $30''$ of a random position and within $3,000 \text{ km s}^{-1}$ of a given redshift near 0.3 ?" The answer, 0.001 before multiplying by the number of tries, is then purported to be "a realistic assessment of the probability of a chance coincidence". While the choice of $3,000 \text{ km s}^{-1}$ can be justified since it follows from the observational uncertainty, the choices of the magnitude limit, -21 , and the separation limit $30''$ are obviously predicated upon the observed values -21.2 and $28''$, respectively, and thus constitute *a posteriori* information.

(2) Another good example is found in a paper by Wolf and Bahcall⁷. They wish to argue that a galaxy in a cluster is physically associated with the N-system Ton256. We quote them as follows: "There appears to be a galaxy about $8''$ from Ton256; the redshifts of the galaxy and Ton256 are identical, to within the observational error of 300 km s^{-1} . The density of galaxies in the central region of the cluster is $\sim 0.08 \text{ galaxies (arc min)}^{-2}$; so the probability of having a galaxy

appear within $8''$ from the QSO, assuming a random distribution of the galaxies near the center of the cluster, is only about 0.4% . We thus assume that the galaxy is really associated with the QSO and is in a bound orbit around the QSO." Again, this is a classic piece of statistical analysis argued *a posteriori*, presumably considered acceptable because the redshifts of the two objects are the same.

In what follows we reconsider the pairs already discovered and then make an *a priori* calculation based on realistic parameters such that a test involving the discovery of further close pairs of QSOs may enable a significant statement to be made.

Let $\Gamma(< m)$ be the sky density of QSOs brighter than m . Then the expected number lying by chance within an angular separation θ of N arbitrary coordinates is

$$\langle n \rangle = 2.4 \times 10^{-7} N \Gamma(< m) \theta^2, \quad (1)$$

with Γ expressed in $(\text{arc deg})^{-2}$ and θ , in arc s. A plot of $\langle n \rangle$ against θ for various $N\Gamma$ is shown in Fig. 1. For N corresponding to the number of QSOs with known redshifts (~ 300), and $\Gamma(m_b < 19.4)$ taken from the Sandage-Luyten survey [$\sim 5 \text{ QSO (arc deg)}^{-2}$], the value for the product $N\Gamma$ is $\sim 1,500 \text{ tries per QSO (arc deg)}^{-2}$. This number could be increased to $\sim 3,000$ by extending the survey to $m_b < 20$. Of course, it is always possible to consider a more restricted sample, yielding a smaller value for $N\Gamma$, but to obtain a value much larger than $3,000$ would require deep plates

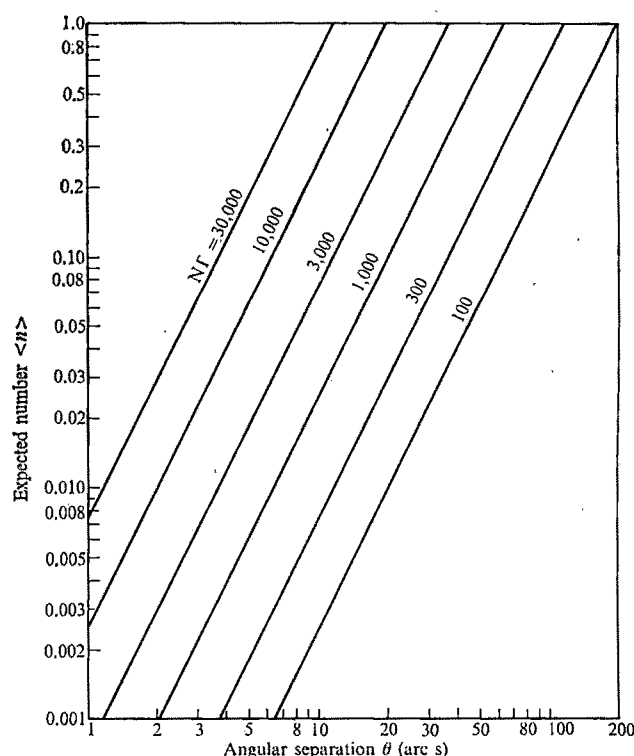


Fig. 1 Dependence of the expected number of apparent pairs upon pre-selected class intervals defined by the maximum angular separation θ to be included and the product of the number of tries N and the sky density $\Gamma(< m)$ of QSOs brighter than the adopted completeness limit m of the sample to be considered.

or deep scans around each of the known QSOs. From a practical point of view it would be difficult to go to $m_n = 21$ on the Palomar Sky Survey because objects at the very limit of the Survey, lying say $5''$ from a brighter object, will tend to be missed and in any case would be very difficult to examine spectroscopically.

The QSO 1548+115a lies about $5''$ from the previously identified QSO 1548+115b (ref. 2). Using the observed separation, the expected number of such pairs, for NT ($m > 19.4$) $\approx 1,500$, is only about ~ 0.01 ; however, since this choice of separation θ is based on *a posteriori* information, the quoted probability is only an approximate lower limit to a realistic *a priori* probability. In order to estimate an upper limit to the *a priori* probability, we can consider the class interval determined by the next nearest separation known—namely, the $35''$ separation of Ton155 and Ton156 (ref. 1). Adopting $\theta = 35''$, the number of pairs with smaller separations expected by chance is ~ 0.4 (see ref. 3).

The statistical significance of the pair 1548+115a,b is thus not well defined; it ranges from $\sim 99\%$ confidence to about 60% . Consequently, the *a priori* statistical significance of this pair cannot be demonstrated. Nevertheless, the proximity of the association is curious, particularly in view of the spectroscopic coincidences already mentioned. Unless additional evidence is uncovered, however, the proximity of 1548+115a,b will remain just a curiosity cited by some as evidence for non-cosmological redshifts, but disregarded by others as a mere coincidence. We therefore propose the statistical test described below.

The vicinity of all ~ 300 QSOs with known redshifts has not been examined closely, and, in fact the method of identification of QSOs from radio positions may have biased the sample against the discovery of close pairs because when a suggested identification close to the radio position is found to be a QSO, no further examination of other nearby objects is usually undertaken. Consequently, more associations may exist (compounding the probabilities). We propose, therefore, that the vicinity around each known QSO be examined for possible conjugates down to $m_n \approx 20$, since it should be feasible to examine any such candidates spectroscopically. For $NT \approx 3,000$, the expected number with chance separations less than $\theta = 10$ arc s is only $\langle n \rangle \approx 0.08$ (see equation 1 and Fig. 1). Thus, the discovery of a second pair of QSOs—or two more pairs of QSOs if the known case 1548+115a,b is excluded from the sample—would be statistically significant at 99.5% confidence, based on *a priori* class intervals.

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Absorption of high energy heavy nuclei and γ rays at the surface of hot neutron stars

SINCE the discovery of pulsars and their association with rotating magnetic neutron stars, it has been realised that pulsars can efficiently accelerate particles to high energies and effectively contribute to the observed cosmic-ray density in the Galaxy. Acceleration of particles to very high energies by the low frequency electromagnetic waves beyond the light cylinder is one mechanism proposed^{1,2}. Others³⁻⁵ have considered the possibility of accelerating particles by the electric field near the surface of the neutron star arising from non-zero $\mathbf{E} \cdot \mathbf{B}$. High energy photons are also likely to be produced in association with these particles and these have been observed from the Crab pulsar. Presumably, younger pulsars may radiate larger numbers of more energetic γ rays.

Though pulsars are potential sources of very high energy particles and γ rays, it is not clear whether the radiation can escape the pulsar environment and reach interstellar space. Various absorption processes can contribute to the destruction of these radiations. Absorption of high energy γ rays in their interactions with the radiation field of the pulsar may occur⁶⁻⁸. Destruction of high energy cosmic rays, especially the photodisintegration of heavy nuclei in the vicinity of pulsars, has also been considered^{9,10}. Heavy nuclei may be destroyed by fragmentation in the high density nebular matter surrounding pulsars in their early stages¹¹. All these processes become important in the early stages of pulsars because of the simultaneous presence of particles of very high energy and large radiation and matter density.

Here I point out that high energy heavy nuclei and γ rays may also be absorbed because of their interactions with the blackbody photons emitted from the surface of the hot neutron star. Neutron stars have surface temperatures greater than 10^7 K when they form and subsequently the temperature decreases¹². Tsuruta *et al.*¹³ have carried out improved cooling calculations taking into account the influence of superfluidity of the matter of the neutron star, the magnetic field and other factors. Their calculations show that for the first 100 yr the surface temperature is 10^7 K and remains greater than 5×10^6 K for another few hundred years for several models. So there is a high density of photons close to the neutron star during the early stages. It is precisely during this period that pulsars lose most of their energy and accelerate particles to high energies. I have discussed the implication of high surface temperature for the radio emission of pulsars elsewhere¹³; here I concentrate on other effects.

If a is the radius of the neutron star and T its surface temperature, the differential spectrum of photons emitted from the surface is

$$n(\epsilon) d\epsilon = \frac{2\epsilon^2}{h^3 c^2} (e^{\epsilon/kT} - 1)^{-1} \text{ photons (cm}^2 \text{ s sr erg)}^{-1} \quad (1)$$

Considering the interaction between a γ ray at O, at a distance R from the centre, travelling radially, and photons from the surface of the neutron star (see Fig. 1) the optical depth for γ - γ interaction is

$$\frac{d\tau}{dR} = \iint (2\pi a^2 \sin \alpha d\alpha \cos \phi) (r^{-2}) \cdot (1 - \cos \beta) n(\epsilon) d\epsilon \frac{\sigma(E_\gamma, \epsilon)}{c} \quad (2)$$

Here $\sigma(E_\gamma, \epsilon)$ is the cross section for pair production in the collision of a γ ray of energy E_γ with a photon of energy ϵ , the term $(2\pi a^2 \sin \alpha d\alpha \cos \phi)$ is the element of area normal to OA, the term r^{-2} represents the inverse square reduction and the term $(1 - \cos \beta)$ takes care of the relative velocity between the two photons. The integration with respect to α

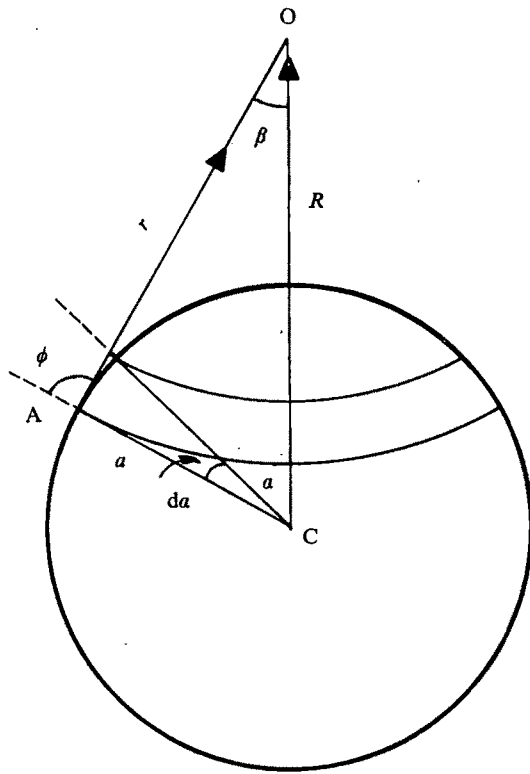


FIG. 1 The interaction between a γ ray or heavy nucleus with blackbody photons from an elemental surface of the neutron star (see text).

is to be carried out between the limits $\alpha = 0$ and $\alpha = \cos^{-1}(a/R)$. The cross section for γ - γ pair production has a threshold $\epsilon E_\gamma(1 - \cos\beta) = 2m^2c^4$. Choosing the variables $x = a/R$, $s = \epsilon E_\gamma(1 - \cos\beta)/2m^2c^4 = s_0 z$ where $s_0 = \epsilon E_\gamma/2m^2c^4$ and $z = (1 - \cos\beta)$, equation (2) becomes

$$\frac{d\tau}{dR} = \iint 2\pi x^2 n(\epsilon) (1/2x^2 s_0^2) \frac{\sigma(s)}{c} s ds \quad (3)$$

Following the treatment of Gould and Schreder¹⁵ I express ϵ in units of kT and introduce the variable $\nu = 2m^2c^4/(E_\gamma kT z_m)$ where $z_m = (1 - \cos\beta_m)$ and $\beta_m = \sin^{-1}(a/R)$. After performing the integration and rewriting the terms in a manner similar to that of Gould and Schreder

$$\frac{d\tau}{dR} = \frac{\alpha^2}{2\pi\Lambda} \left(\frac{kT}{mc^2}\right)^3 [1 - (1 - x^2)^{1/2}]^2 f(\nu)$$

where $\alpha = e^2/\hbar c$, $\Lambda = \hbar/mc$ and $f(\nu)$ is the function introduced by Gould and Schreder, who give a plot of $f(\nu)$ against ν . The function $f(\nu)$ has a maximum value of ~ 1 at $\nu \simeq 1$. The total optical depth for γ - γ collision is

$$\begin{aligned} \tau_{\gamma\gamma} &= \int \frac{d\tau}{dR} dR = \frac{\alpha^2}{2\pi\Lambda} \left(\frac{kT}{mc^2}\right)^3 a \\ &\cdot \int_1^0 [1 - (1 - x^2)^{1/2}]^2 f(\nu) \frac{dx}{x^2} \quad (4) \\ &= 1.2 \times 10^3 T_\gamma^3 I_{\gamma\gamma}(\nu) \quad (5) \end{aligned}$$

where $I_{\gamma\gamma}(\nu)$ is the integral in equation (4), T_γ is the surface temperature in units of 10^7 K and a has been taken as 10^6 cm. I have evaluated $I_{\gamma\gamma}(\nu)$ numerically and Fig. 2 shows a plot of $\tau_{\gamma\gamma} T_\gamma^{-3}$ as a function of $T_\gamma E_\gamma$ (GeV).

Photodisintegration of heavy nucleus is most effective in the giant resonance region of photon energy 10 to 20 MeV in the rest system of the nucleus. The cross section increases as $A^{2/3}$ where A is the mass number of the nucleus. Here I shall

consider the disintegration of Fe nuclei. The photonuclear cross section can be written as¹⁶

$$\sigma(y) = c(y - 1) \exp[-(b(y - 1))] \quad (6)$$

where b and c are constants and $y = s/p$, p being the threshold energy for the reaction and $s = (\gamma\epsilon)(1 - \cos\beta)$ $s_0 z$ is the energy of the blackbody photon in the rest system of the nucleus of Lorentz factor γ . Going through a similar treatment as for γ - γ collisions and introducing similar variables I arrive at the following expression for $\tau_{\gamma H}$, the optical depth for disintegration of heavy nuclei:

$$\tau_{\gamma H} = 2\pi\sigma_0 \left(\frac{kT}{\hbar c}\right)^3 a I_{\gamma H}(\nu) \quad (7)$$

where

$$I_{\gamma H}(\nu) = \int_1^0 [1 - (1 - x^2)^{1/2}]^2 f_H(\nu) dx/x^2,$$

$$\nu = p/\gamma k T z_m$$

$$f_H(\nu) = \nu^2 \int_\nu^\infty (e^\epsilon - 1)^{-1} \phi(\epsilon/\nu) d\epsilon,$$

$$\phi(y) = \int_1^y y \bar{\sigma}(y) dy, \text{ and}$$

$$\bar{\sigma}(y) = \sigma(y)/\sigma_0 = \sigma(y)/(10^{-25} \text{ cm}^2)$$

For photodisintegration of Fe nuclei, the cross section can be represented as

$$\bar{\sigma}(y) = 11(y - 1) \exp[-2.7(y - 1)]$$

with a threshold of 13 MeV and a maximum cross section of 150 mb at 18 MeV. With this parametrisation for the cross section, the integral $I_{\gamma H}$ has been numerically evaluated and τ_γ (Fe) calculated. In Fig. 2 I show the plot of τ_γ (Fe T_γ^{-3}) as a function of γT_γ .

The total optical depth for γ - γ collision is shown in Fig. 3 for three different values of temperature. For all temperatures $> 2 \times 10^6$ K, there is a wide range of energies in which

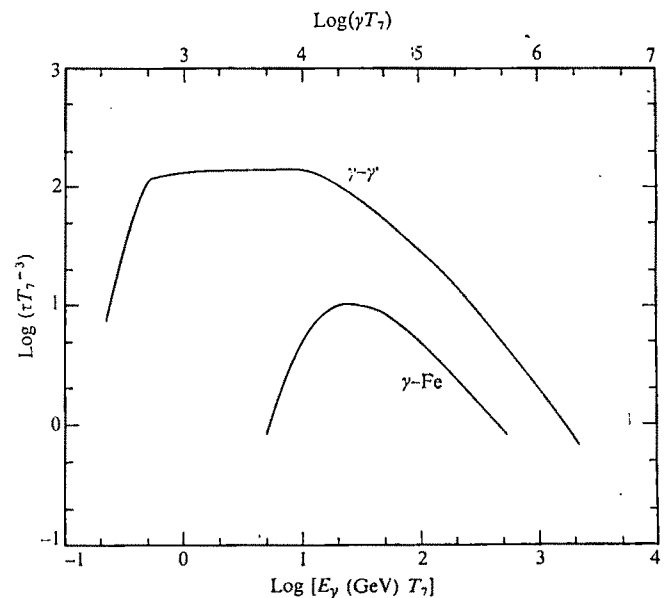


FIG. 2 The quantity $\tau_\gamma T_\gamma^{-3}$ plotted as a function of $E_\gamma T_\gamma$. Here τ is the optical depth for γ - γ and γ -Fe interaction, T_γ is the surface temperature in units of 10^7 K, γ is the Lorentz factor of the Fe nucleus and E_γ the photon energy in GeV.

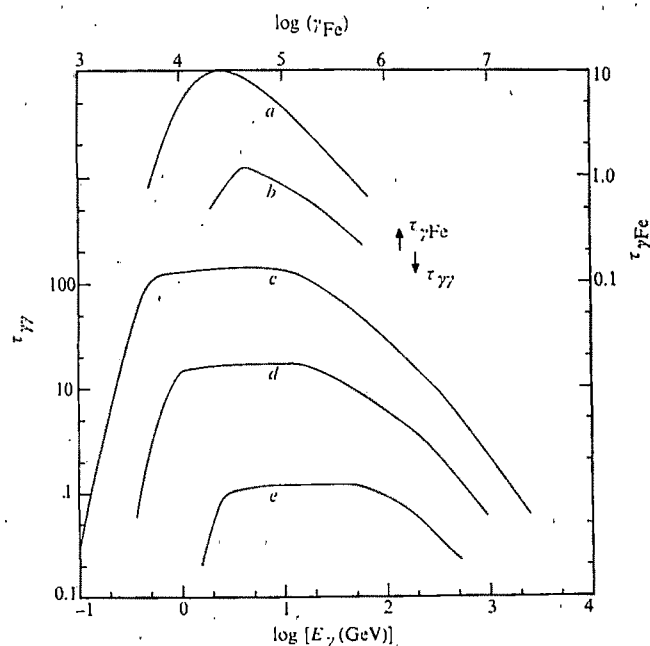


FIG. 3 Optical depth for $\gamma\text{-}\gamma$ interaction (bottom and left scales) and $\gamma\text{-Fe}$ (top and right scales) plotted as a function of energy for different temperatures. Legend as in Fig. 2. a, 10^7 K; b, 5×10^6 K; c, 10^7 K; d, 5×10^6 K; e, 2×10^6 K.

$\tau_{\gamma\gamma} > 1$. The larger the temperature the higher is the optical depth and wider the range of energy for absorption. In the first few hundred years of a pulsar's life, the temperature is greater than 5×10^6 K, leading to the absorption of γ rays of energy ranging from a few hundred MeV to about 1 TeV. When the temperature is 10^7 K the lower limit extends to about 200 MeV. High energy γ rays can also be absorbed because of pair production in the magnetic field of the pulsar. This has been investigated by Sturrock⁴ who finds that for a surface magnetic field of 10^{12} gauss, this process is important for $E_\gamma \gtrsim 1$ GeV. The absorption mechanisms discussed in this paper not only extends to lower energies but is also independent of the magnetic field (as well as of the beamed radiation that may be associated with the pulsar).

The optical depth for photodisintegration of Fe nuclei is also shown in Fig. 3 for two different temperatures. Here also the optical depth > 1 for $T > 5 \times 10^6$ K over a reasonably wide range of energies. But the range of energies over which absorption occurs is narrower than that for γ rays. In the first hundred years, the surface temperature of the neutron star is $\sim 10^7$ K. It is during this time that the pulsar loses most of its energy and also accelerates particles up to 10^{18} to 10^{19} eV. The highest energies will not be affected by photodisintegration (Fig. 3); but in the range of a few times 10^{12} eV per nucleon to a few times 10^{14} eV per nucleon, Fe nuclei will be disintegrated preferentially. The medium nuclei will be affected less and over a narrower range of energies, since the photonuclear cross section for them is low by a factor about 2.5. Thus, the cosmic-ray nuclei escaping from the pulsar surface will undergo changes in spectra and composition. Such changes have been observed for cosmic rays with energies of tens of GeV^{17,18}. If a large fraction of cosmic rays come from acceleration processes close to the surface of the neutron star, the above mechanism will induce spectral and compositional changes in the range of 10^{12} eV per nucleon to 10^{14} eV per nucleon.

Smoluchowski¹⁹ has pointed out that because of the anisotropic conductivity of neutron star matter, the surface temperature at the poles can be a factor of 2 to 3 higher than the equatorial temperature. For the absorption processes discussed here, it is the polar temperature which is more

relevant; this being higher than the mean temperature used, the absorption may be more severe than indicated.

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Duration of dMe flares

GERSHBERG and Shakhovskaya¹ have suggested that the time scales of flare development depend principally on the peak amplitude of flare events, implying that stronger events last longer. They support their contention with observations taken from eleven flare stars. In an earlier investigation of flare decay rates I concluded that time scales are intrinsic properties of the stars themselves².

The relationship determined by Gershberg and Shakhovskaya from their data takes the form

$$\log_{10} \left(-\frac{dL}{dt} \right)_1 = 0.75 \log_{10} L_{\text{peak}} + \text{constant} \quad (1)$$

where $(-dL/dt)_1$ is the decay rate on the light curve chosen in a uniform manner and L_{peak} is the peak radiation loss. The corresponding relationship derived by myself, and put in the same form, is

$$\log_{10} \left(-\frac{dL}{dt} \right)_K = \log_{10} L_{\text{peak}} + AM_{\text{bol}} + \text{constant} \quad (2)$$

where M_{bol} is the stellar bolometric absolute magnitude and A is a constant very nearly equal to 0.2.

For any given star the observable range of the magnitude of peak event is constrained by the detection threshold of the instruments used and by flare incidence statistics, so that the less energetic flares are observed preferentially on

TABLE 1 Data sets for determining time scale properties in the flares of UV Ceti

Epoch	Telescope	No. of flares	U_{det}	U_{cutoff}	No. of flares used
1966.9	91 cm	143	14.6	13.6	25
1967.65	91 cm	38	15.5	14.5	9
1967.77	60 cm	152	14.2	13.2	29
1968.76	91 cm	107	15.6	14.6	35

TABLE 2 Data sets for determining time scale properties in the flares of Wolf 630 and CoD-31°17815

Star	M_V	Epoch	Telescope	Total No. of flares	U_{det}	U_{cutoff}	No. of flares used
Wolf 630	10.8	1968.4	91 cm	24	15.0	14.4	13
		1969.5	60 cm	26	14.6	14.0	10
		1970.5	152 cm	61	15.2	15.0	42
CoD-31°17815	8.9	1970.5	152 cm	31	15.0	14.8	21

stars of lower luminosity (ref. 3 and Fig. 3 in ref. 1). Because of these effects a biased result is difficult to avoid unless care is taken. Figures 1 and 2 of Gershberg and Shakhovskaya¹ show this effect: the flares of any given star are found in restricted portions of the diagram. Furthermore, because the intrinsic scatter of the parameters which describe flares is large^{2,3}, the relationship presented by these authors could not have been constructed with adequate confidence from observations of a single star. Equation (2) can be made to fit the data of Gershberg and Shakhovskaya as a direct result.

To decide between the alternatives the following two tests are proposed. First, the relationship between peak flare amplitudes and a time scale parameter for the star with the largest homogeneous set of observations is examined. For this purpose my observations^{3,4} of UV Ceti covering the range $9 < U_{peak} < 14.9$ were used. Subtracting $\log_{10} L_{peak}$ from both sides of equation (1), the equation of Gershberg and Shakhovskaya can be written

$$\log_{10} \frac{dU}{dt} = 0.108 U_{peak} + \text{constant} \quad (3)$$

In place of the decay rate, the most densely sampled time scale parameter which I use is the mean duration of half peak light, $T_{0.5}$; other time scale parameters were not measurable for as many of the detected flares. The mean duration and decay rate are inversely related and equation

(3) can therefore be written

$$\log_{10} T_{0.5} = \alpha U_{peak} + \text{constant} \quad (4)$$

where α is -0.108 (ref. 1), or zero (ref. 2).

Table 1 presents the data examined using least squares. The faint cutoff level has been selected 1 mag brighter than the adopted detection limit for the data sets in order to minimise effects of incomplete sampling at the faint event threshold³. Setting U_{cutoff} 0.5 mag fainter altered the value of α by less than one standard error. The values of the coefficient for both instances are

$$\alpha = -0.005 \pm 0.021, \quad 98 \text{ events}$$

$$\alpha = +0.012 \pm 0.025, \quad 132 \text{ events}$$

respectively.

The second test compares the time scales of events of comparable absolute peak energy for stars of different luminosities. Data for Wolf 630 and CoD-31°17815 (ref. 3) suitable for comparison with those of UV Ceti are presented in Table 2. A sufficient range of peak event magnitudes was observed in Wolf 630 to yield a usable value of the coefficient α (-0.012 ± 0.011). As in the case of UV Ceti, there seems to be no significant relationship between the time scales of flares, and peak amplitudes. Because of this, CoD-31°17815 is the most luminous star used in the proposed comparison, even though the observed range of peak magnitudes of events is still too small to allow a reliable determination of the coefficient α .

Figure 1 presents all the data used in this investigation. A correlation analogous to that described by Gershberg and Shakhovskaya is apparent at first glance: the longer time scales are found in the stronger events. But the weakest events detectable for each star depend strongly on stellar luminosity, colour and parallax, and so the effect is deceptive. For the three stars taken together the data are complete only for flares brighter than $M_{V,peak} = 15.06$. If only this portion of the diagram is considered, the apparent correlation disappears completely. Comparing mean time scales of events lying in the range $14.0 < M_{V,peak} < 15.0$, the flares of shortest duration are those of UV Ceti, and the longest lasting are those of CoD-31°17815. The result is shown in Table 3. From these results it is difficult to conclude that the event durations are distinguishable by their peak amplitudes. But they correlate with stellar absolute magnitude as found by Haro and Chavira⁵. A linear relationship describing the effect, is

$$\log_{10} T_{0.5} = 1.084 - 0.119 M_V \quad (5)$$

The analogous equation of Gershberg and Shakhovskaya¹ yields values for $\log_{10} T_{0.5}$ of -0.81 , -0.41 and -0.24 , for UV Ceti, Wolf 630 and CoD-31°17815, respectively.

Gershberg and Shakhovskaya do not explicitly state that their results apply to flare phenomena on isolated stars and indeed the present analysis indicates that no such relationship applies to isolated flare stars: flare durations are not correlated with peak amplitudes. Even if a relationship of the sort proposed by Gershberg and Shakhovskaya had been found, it would still not have precluded a relationship between flare time scales and stellar luminosity: a comparison

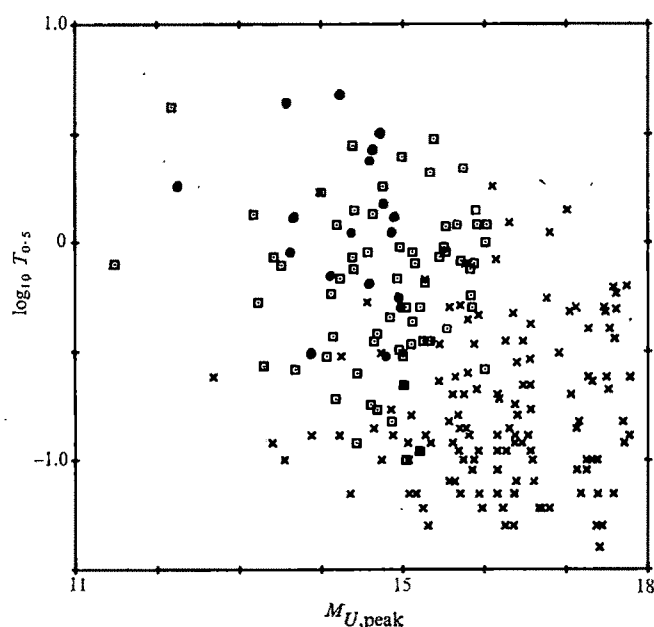


FIG. 1 Relationship between flare durations at half peak light, $T_{0.5}$ (ordinate) and peak light, on a scale of absolute magnitude, $M_{V,peak}$. \times , UV Ceti; \square , Wolf 630; \bullet , CoD-31°17815. The apparent linear correlation between peak light and mean duration is a selection effect. The data are complete only for events brighter than $M_{V,peak} = 15$. This sector shows no correlation between peak light and event duration.

TABLE 3 Mean values of the event duration parameter $\log_{10} T_{0.5}$ for $14 < M_{U, \text{peak}} < 15$

Star	M_V	$\log_{10} T_{0.5}$	$10^{\log_{10} T_{0.5}}$ (min)	No. of flares
UV Ceti	15.5*	-0.74 ± 0.09	0.184	10
Wolf 630	10.8*	-0.26 ± 0.07	0.552	31
CoD-31 ⁹ 17815	8.9	$+0.07 \pm 0.10$	1.29	13

* Value per component, assuming equal luminosity.

of flares of different stars, which fall in the same range of absolute peak energy levels, shows a strong correlation between the mean event duration and stellar luminosity. In conclusion, it seems that the interpretation of the Haro-Chavira phenomenon proposed by these authors is less appropriate than my interpretation².

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Internal origin for lunar rilled craters and the maria?

THE origin of lunar craters has been attributed to both volcanism¹⁻³ and/or impact⁴⁻⁶, although since the return of the Apollo 11 volcanic rocks opinion has favoured an endogenic origin for a greater part of the lunar morphology than was previously accepted⁷.

Evidence for lunar vertical crustal movement is provided by Apollo 15 photographs of dipping structures on Silver Spur (25° 40' N, 3° 58' E)⁸ and by craters containing rilles indicating either uplift⁹ or subsidence¹⁰.

Principal lineaments such as rilles and faults, and craters larger than 10 km containing internal rilles, were drawn on overlays of Orbiter I, II, III, IV and V high and low resolution photographs and transferred to a Mercator projection map (Figs 1 and 2) of the Moon.

Because the morphology of the rilles and their enclosing craters is varied, the internally rilled craters can be divided into a number of subgroups (Fig. 3), by shape and size of the enclosing crater and by the rille pattern on the crater floor.

Small craters are less than 15 km in diameter and the diameter of the floor is less than the rim radius. The large craters are generally greater than 15 km in diameter with the diameter of the floor equal to, or much larger than, the rim radius.

On the near side of the Moon, craters of types I, II and III are situated predominantly around the maria, particularly around the western edge of Oceanus Procellarum, with most situated between the maria and the outer limit of the parallel-sided flat-floored normal rilles (see Figs 1 and 2).

There are too few type IV craters for their distribution pattern to be significant but the type V craters, apart from being found in the vicinity, of type I, II and IV, are also found along the major rifts such as the Rheita Valley. A number are also found within the larger flat-bottomed craters

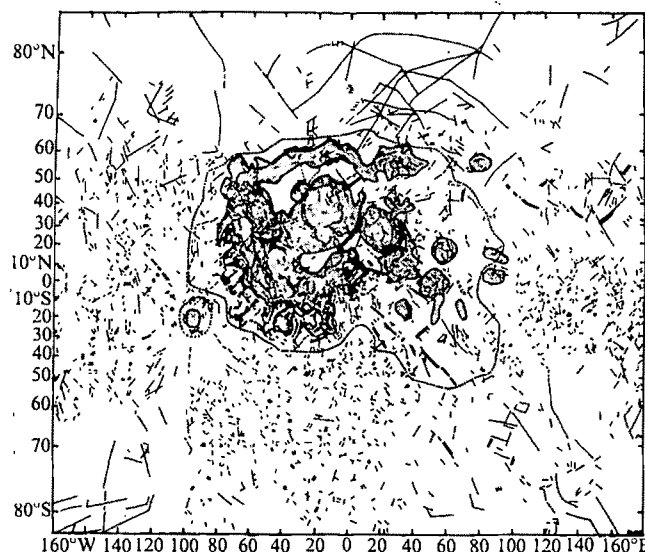


FIG. 1 Map of major lineaments on the Moon; maria have bold outline, dotted line encloses 'normal' rilles.

which themselves contain internal rilles, for example Einstein.

Whereas there are approximately 300 internally rilled craters on the near side of the Moon, there are only about forty on the far side. Although photographic coverage and resolution of the near side is considerably better than that of the far side, photography of the far side is such that only small craters of those occurring in restricted areas of oblique or non-coverage could be added.

A few of the craters on the far side are associated with maria (Komarov) and some are associated with larger craters (Jules Verne and Poincaré groups). The two large craters Oppenheimer and Schrödinger are also rilled. Small rilled craters are again found within larger flat-floored craters (Aitken, Barbier, and Pannekoek). A few internally rilled craters approximately 40 km in diameter (Hogg and Hutton) do not conform to any apparent distribution pattern.

A depth/diameter plot (Fig. 4) of a number of the rilled craters of types I, II and III on the near side (using data from the Arthur catalogue¹¹⁻¹⁴) shows that the majority are wider than the sharply sculptured, well defined, generally deep 'eumorphic' craters which are represented by the line on the diagram (taken from ref. 15).

Because rilles in older craters are much more subdued than those of younger craters, it can be assumed that the rilles in all internally rilled craters formed at about the same time as the crater floor and not as a result of deformation caused by the proximity of the maria.

Figure 4 shows that rilled craters of Copernican, Eratosthenian and pre-Imbrian ages occur in groups approximately parallel to the line for eumorphic craters, with the progressively older craters plotting further toward a smaller depth/diameter ratio. The craters of Imbrian age, however, occur within the fields of all the other groups. This scatter of craters of Imbrian age could be attributed to error in depth/diameter measurements, incorrect identification, or to the fact that the Imbrian craters were produced differently from those of other periods. It is unlikely that the Arthur catalogue is biased toward Imbrian craters alone and the estimated errors for these craters are insufficient to bring the craters into a restricted group on the plot. The Imbrian craters form the basis of the United States Geological Survey lunar stratigraphy so an incorrect age notation is unlikely.

Thus the difference between Imbrian craters and those of other periods is probably due to a peculiarity in the formation of the former. This could have been because vast quantities of lavas were erupted onto the lunar surface during the Imbrian period and the physical constitution of the Moon

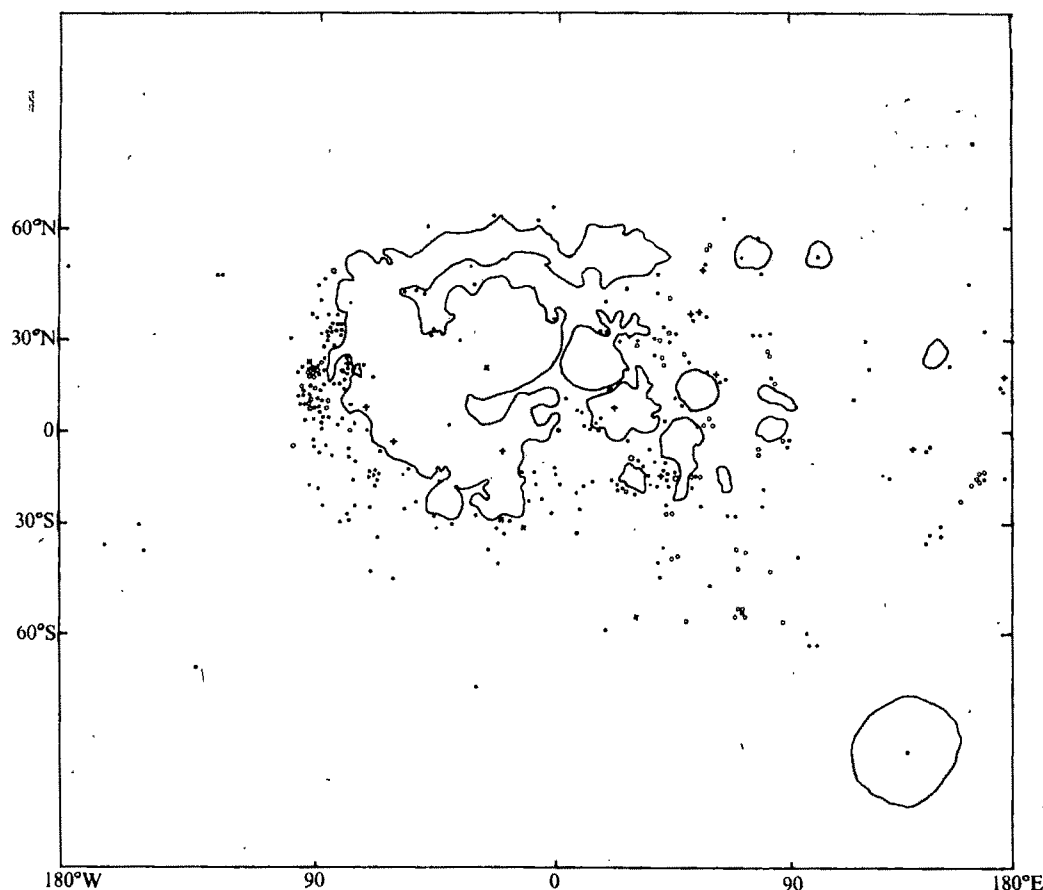


FIG. 2 Distribution map of rilled craters on the Moon; ●, type I; ■, type II; X, type III; +, type IV; ○, type V.

at this time may have been radically different from that of earlier and later periods.

Another problem associated with the origin of the rilled craters is that they occur adjacent to grossly similar craters without rilles. Possible explanations for this are that either the rilles in non-rilled craters are covered by later deposits, or that structural defects in the crust beneath rilled craters may have caused preferential rille development. Counts of craters larger than 1 mm within both rilled and non-rilled craters were carried out on the high resolution Orbiter photographs. The results (Fig. 5) show that in most cases the floors of the non-rilled craters have a higher crater density than the floors of the rilled craters in the same age group, arguing against the possibility of a younger floor covering the rilles. The greater crater density may, however, be due to a high percentage of small endogenic craters.

The simplest explanation for rilled and non-rilled craters is that they have tectonically different origins. If the craters are of impact origin, the rilled craters may have been deeper, or have formed in structurally weaker material which allowed a greater degree of isostatic readjustment after infilling, producing the rilles. If, on the other hand, the craters are of endogenic origin the rilles could have been produced by

either updoming of a diapiric body or subsidence after the evacuation of a magma chamber.

The geographical distribution (Fig. 2) of types I, II and III rilled craters indicates that their formation was also

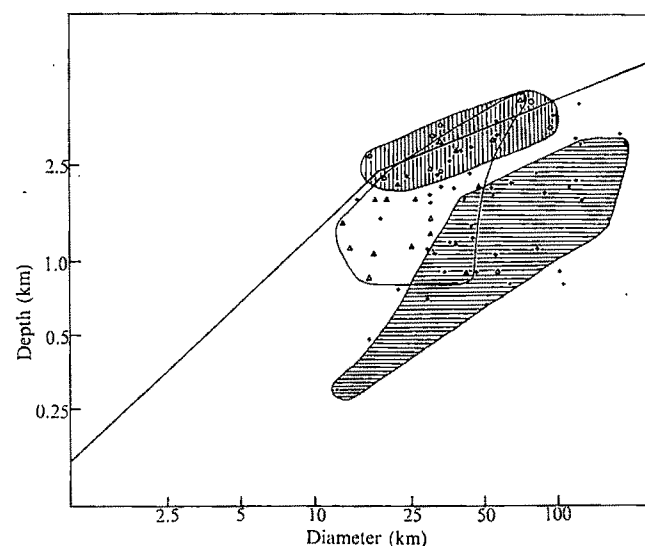


FIG. 4 Depth/diameter graphs of some rilled craters on the near side; horizontal ruling, pre-Imbrian; vertical ruling, Copernican; unshaded outline, Eratosthenian; ○, Copernican; ▲, Eratosthenian; +, Imbrian; ●, pre-Imbrian.

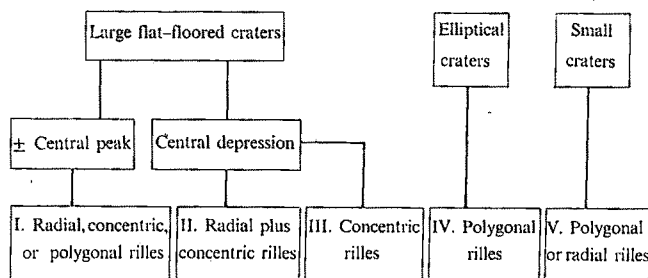


FIG. 3 Classification of rilled craters.

related to the maria. A number of hypotheses can be advanced to account for this relationship depending on whether: (a) both the internally rilled craters and mare basins are of external origin; (b) the craters are of internal origin and the mare basins were formed by impact; (c) the mare basins are of internal origin and the craters were formed by impact; (d) they are both of internal origin.

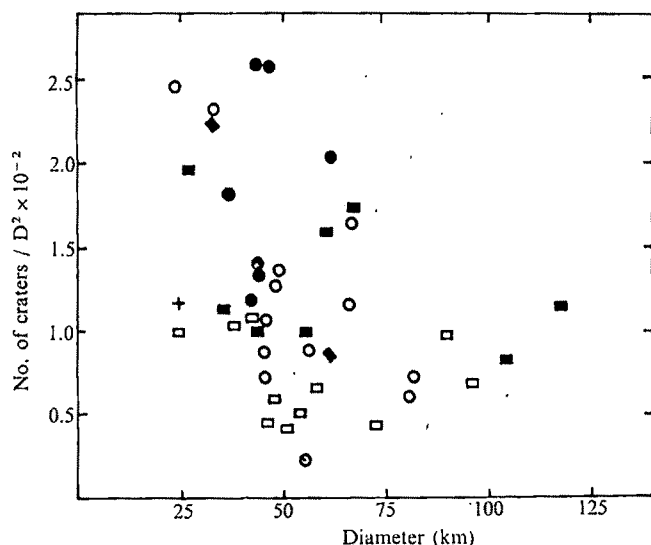


FIG. 5 Crater density plot for small craters inside rilled and non-rilled craters. Subdivided on age taken from refs 11-14. Rilled: ● = 3; ○ = 2; ◐ = 1. Non-rilled: ◆ = 4; □ = 3; + = 2; + = 1. D, Diameter.

(a) If the rilles within pre-Imbrian rilled craters are of pre-Imbrian age, this first hypothesis is unlikely since bodies would have to have struck the Moon during the pre-Imbrian period within the limits of the then non-existent normal rilles. (These impacts would have been followed by much larger impacts forming the mare basins within the area defined by the pre-Imbrian impacts but not covering such a large geographical area of the Moon. These in turn were followed by smaller bodies which were preserved immediately around the edges of the maria but became swallowed up in the maria. The whole argument sounds very unconvincing.)

(b) If it is argued that the rilled craters are of internal origin as a result of their proximity to the maria, the presence of pre-Imbrian rilled craters would be related to, and surround, an impact site that did not yet exist.

(c) Arguments against this hypothesis are similar to those of (a) and (b) in that it is very difficult to explain the observed crater distributions.

(d) If the mare basins are of internal origin, the distribution of the rilled craters can be readily explained as resulting from their situation in areas of lunar crustal weakness which on the near side of the Moon were centred on the maria. Internal magmatic processes originating in the pre-Imbrian period reached a maximum during the Imbrian period with the formation of the maria by lava eruption and block subsidence. This internal activity steadily declined and became confined to the areas surrounding the maria where normal rilles, mare terraces and rilled craters formed as a result of crustal uplift and subsidence. The differences between the Imbrian rilled craters and those of other periods could be a result of the peculiar state of the lunar crust during the outpouring the mare lavas.

The rilled craters on the far side of the Moon can be similarly interpreted as occurring in areas of lunar crustal weakness. Because the crust is thicker on that side of the Moon¹⁶, either the magma supply was insufficient or magma could not be supplied to the surface at sufficient speeds to produce extensive maria.

It is not necessary to propose an impact origin to account for the areas of weak crust. Structural effects, such as fracturing and faulting, associated with the formation of the lunar grid of Moonwide extent (ref. 2, and G. Fielder and J. L. Whitford-Stark, to be published) could have produced these weaknesses.

The rilled structures in the types IV and V craters could be of volcanic origin; either by cooling contraction of a lava

lake or tholoid doming. Other evidence for their being of internal origin is that many are situated along probable faults, such as the Rheita Valley.

To summarise, if the rilles in large rilled craters are the same age as the enclosing crater, the distribution of the craters precludes them from being anything other than of internal origin and, as a consequence, the maria and their basins must also be of internal origin. The types IV and V craters are also probably related to volcanic processes.

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Photo-induced free radicals on a simulated Martian surface

THE atmospheric pressure at the surface of Mars is about 5.5 mbar and the atmosphere is composed mainly of CO₂ with trace amounts of CO, O₂ and H₂O (refs 1 and 2). The Martian dust has an intermediate SiO₂ content of 60 ± 10% (ref. 3). Although it has been thought that the synthesis in a planetary atmosphere of organic compounds relevant to the origin of life requires chemically reducing conditions, Hubbard and colleagues^{4,5} have reported the synthesis of organic compounds by ultraviolet irradiation (λ > 250 nm) of a solid with a gas mixture adsorbed which is compositionally similar to the oxidised atmosphere of Mars. Since the question whether organic compounds could have been formed on the surface of Mars is of fundamental importance to the concept of chemical evolution and to the interpretation of results from the future Mars Viking Mission molecular analysis experiment, we have attempted to confirm these results while concentrating on identifying the primary process and the reactive intermediates involved. Here we describe the electron spin resonance (ESR) study of free radicals in the ultraviolet irradiation of a simulated Martian surface. (A preliminary account was presented at the Fourth International Conference on the Origin of Life, Barcelona, Spain, June 1973.)

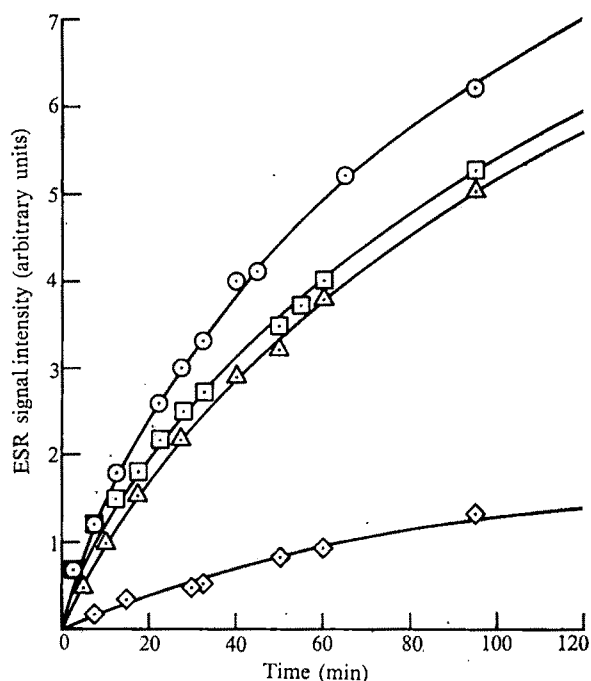


Fig. 1 Formation of CO_2H radicals produced at -170°C by ultraviolet irradiation of CO (15 Torr), CO_2 (15 mmHg), CO (15 mmHg) + CO_2 (135 mmHg), and CO (15 mmHg) + CO_2 (135 mmHg) + O_2 (1 mmHg) adsorbed on silica gel. ○, CO; □, CO + CO_2 ; △, CO + CO_2 + O_2 ; ◇, CO_2 .

The silica gel used in these experiments consisted of 60 to 80 mesh, acid-washed gas chromatograph grade powder. The CO (free of iron carbonyl), CO_2 and O_2 used were Matheson high quality research grade. The vacuum system used to introduce gases had had no previous exposure to mercury. In a typical experiment, the silica gel (200 mg) was placed in an ESR tube (made of Suprasil, 20×0.4 cm outer diameter) and was heated at 180°C for 6 h in a vacuum of 10^{-5} mmHg, then continuously evacuated for 16 h at room temperature. Various gases were then introduced through the vacuum line into the ESR tube and exposed to the silica surfaces. The resultant sample, in the sealed ESR tube, was then placed in an ESR cavity and irradiated with a low pressure mercury lamp through a Vycor filter. Thus the ultraviolet light used for irradiations contained, chiefly, the 253.7 nm line with minor components in the longer wavelength region. Irradiation was usually carried out at -170°C . The ESR spectra were recorded on a Varian V-4500 X-band spectrometer equipped with a variable temperature attachment. The g values were obtained by comparison with the standard value for diphenylpicryl hydrazyl (DPPH, $g = 2.0037$). The estimated error for each g value was about ± 0.0002 . The vapour pressures for all gases indicated below were those observed at room temperature with a calibrated thermal conductivity gauge. These values were arbitrarily chosen for experimental convenience; nevertheless, CO_2 was kept in excess in the systems containing a mixture of gases.

Irradiation of the silica gel alone at -170°C slowly produced a radical species, as evidenced by the appearance of the ESR signal. The spectrum consists of a major peak with $g = 2.0120$ and a minor peak at a field 27 gauss lower ($g = 2.0292$). The g value of the major peak is consistent with the reported g value (2.01) for the OH radical produced in a frozen H_2O medium at -196°C (ref. 6). The same ESR spectrum was obtained more quickly when vapour from dilute aqueous H_2O_2 (vapour pressure, 0.3 mmHg) adsorbed on silica gel was irradiated at -170°C . Both radicals showed the same thermal decay behaviour; the ESR signals disappeared when the samples were warmed to room temperature. Since photolysis of H_2O_2 with 253.7 nm light results only in OH formation⁷, we conclude that this major peak is due

to the OH radical. Exposure of the silica gel to H_2O vapour (5 mmHg) followed by irradiation at -170°C did not lead to enhanced signal intensity due to additional OH radical formation.

When the silica gel was exposed to O_2 (1 mmHg) and then irradiated at -170°C , a new radical species was formed. The spectrum shows g value anisotropy having $g_x = 2.0255$, $g_y = 2.0088$, and $g_z = 2.0037$. It also shows doublet hyperfine splitting of 10 gauss for the peak at g_x because of coupling to a proton. This spectrum is identical with that of the O_2H radical reported by Smith and Wyard⁸.

When a silica gel sample containing CO (15 mmHg), or CO_2 (15 mmHg), or a mixture of CO (15 mmHg) and CO_2 (135 mmHg) was irradiated at -170°C , a different radical was observed. The signal appears at a higher field than the OH radical, and the line shape shows characteristic g -value anisotropy. The g values for this new radical are $g_x = g_y = 2.0024$ and $g_z = 1.9971$, and its isotropic or average g value is 2.0006, which is close to the isotropic liquid g value of 2.0002 reported for the CO_2H radical⁹, and which is in excellent agreement with the average g -value of 2.0005 attributed by Chantry *et al.*¹⁰ to the CO_2H radical formed by gamma irradiation of potassium bicarbonate crystals. Furthermore, the average g value (2.0006) of this radical is below the free spin value (2.0023), indicating that it is unlikely to be the π radical, HCO_2 . But it is consistent with the σ radical, CO_2H , in which the unpaired spin resides on an orbital of approximately sp^2 type¹¹. The g anisotropy of the CO_2H radical indicates that it was formed on the silica surface where it was stable for hours in the dark at -170°C . On warming it decayed rapidly and the ESR spectrum disappeared at about -50°C . The CO_2H radical was the only radical product observed at -170°C when a mixture of O_2 (1 mmHg), CO (15 mmHg), and CO_2 (135 mmHg) adsorbed on silica gel was irradiated. The relative yields of the CO_2H radical obtained under various conditions as estimated from ESR signal intensity are shown in Fig. 1. No ESR signal was observed when CO, CO + H_2O , CO + CO_2 , or CO + CO_2 + O_2 was irradiated at -170°C in the absence of silica gel.

After warming to room temperature, continued irradiation of all previously irradiated samples (CO, CO_2 , CO + CO_2 or CO + CO_2 + O_2 adsorbed on silica gel) resulted in the generation of a third radical species. The line width is narrower (3 gauss, compared with 5 gauss for CO_2H). Furthermore, this radical was stable at room temperature for days, thus, indicating that a new radical was formed. The g values for this radical are $g_x = g_y = 2.0024$ and $g_z = 1.9979$. Comparison of the ESR line shape, line width and g values of this radical with other reported data^{12,13} reveals that this is the carbon dioxide anion radical, CO_2^- . When HCO_2H was adsorbed on silica gel and irradiated at room temperature, an identical ESR spectrum was obtained. These two radicals also show the same thermal decay behaviour.

Results of the present ESR study suggest that the ultraviolet photolysis of CO or CO_2 , or a mixture of CO and CO_2 adsorbed on silica gel at -170°C , involves the formation of OH radicals and possibly H atoms as the primary process, followed by the formation of CO_2H radicals. Although other radicals might also have been formed, microwave power saturation and thermal decay experiments show that the indicated radicals were the principal radical products. The H atoms and OH radicals may be produced by: (i) direct photodissociation of H_2O vapour and/or H_2O physically adsorbed on silica gel; (ii) absorption of the ultraviolet excitation energy by the silica surface with subsequent cleavage of the silanol bonds, ($=\text{Si}-\text{H}$ and $=\text{Si}-\text{OH}$); (iii) energy transfer from silica gel to adsorbed H_2O followed by cleavage of $\text{H}-\text{OH}$ bond; or (iv) a combination of the above. Process (i) seems very unlikely since ultraviolet irradiation was carried out with the wavelength (>253.7 nm) longer than that absorbed by H_2O molecules. Moreover, no ESR signal was observed when H_2O ad-

sorbed on NaCl was irradiated at -170°C with the same light source. In the NaCl system, only H_2O vapour and physically adsorbed H_2O were present. Furthermore, no ESR signal was observed when a mixture of CO (15 mmHg), CO_2 (135 mmHg) and H_2O (1 mmHg) adsorbed on NaCl was irradiated at -170°C . We therefore conclude that either (ii) or (iii) or (iv) is more likely. Similar processes such as photosensitisation of hydrogen dissociation by silica gel¹⁴, and ultraviolet-induced bond cleavages of =Si-OCH_3 and =SiO-CH_3 (ref. 15) have been reported. The energy transfer process (iii) may be of minor importance relative to cleavage of silanol bonds (ii) since there was no significant increase in the ESR signal due to OH radicals during the irradiation of silica gel containing 5 mmHg of H_2O vapour. When silica gel preheated at 450°C (instead of 150°C) was irradiated at -170°C , only a very weak ESR signal of the OH radical was observed. Apparently, at this temperature, most of the silanol groups condensed to liberate H_2O , leaving surface siloxane groups¹⁶.

H atoms were not observed by ESR in our experiments, possibly because of their low concentration and/or rapid combination to form H_2 molecules. Reactions of the H atom and OH radical with CO can form the HCO radical and CO_2H radical, respectively. CO is an efficient scavenger for the OH radical¹⁷; therefore, the formation of CO_2H radical is expected to be favoured. Presumably because of the low concentration of free H atoms available for capture by CO, the CHO radical could not be detected under the conditions of these experiments. In fact, Hubbard *et al.*^{4,5} observed only a very low yield of HCHO as compared to HCO_2H . When CO_2 is adsorbed on silica gel, photochemically produced H atoms could react with CO_2 and reduce it to CO_2H . But reaction of OH radicals with trace amounts of CO present in CO_2 as impurity might also contribute to the formation of CO_2H radicals. For the mixture of CO and CO_2 on silica gel, both gases were adsorbed on the surface, so the sites available for CO adsorption would be decreased. Consequently, the total amounts of CO_2H radical formed would be lower than when CO alone was adsorbed on silica gel (Fig. 1).

The formation of HCO_2H from CO_2H radicals was demonstrated by the irradiation experiment at room temperature. After the CO_2H radicals had disappeared upon warming, re-irradiation of the silica gel sample at room temperature produced CO_2^- radicals. This same radical was also observed when authentic HCO_2H adsorbed on silica gel was irradiated at room temperature. Apparently, HCO_2H was formed during the thermal decay of CO_2H radicals, and irradiation at room temperature resulted in the photodecomposition of HCO_2H .

This study confirms the reports of Hubbard *et al.* that HCO_2H and possibly HCHO and other organic compounds were formed during the photolysis of atmospheric gases adsorbed on a simulated Martian surface, suggesting that these compounds may be formed on the surface of Mars as well. An important observation in this study is that these organic compounds were formed by the reaction of free radicals generated by the ultraviolet irradiation, with OH radicals playing a very important role. This observation is in contrast to the mechanism proposed by Hubbard *et al.*, who suggest that CO excitation is responsible for the organic synthesis. Our study suggests that photochemical synthesis of organic compounds could occur on Mars if the siliceous surface dust contains enough silanol groups and/or adsorbed H_2O , as bound water. Houck *et al.*¹⁸ recently provided high altitude infrared spectroscopic evidence that the wind-blown surface dust on Mars contains about 1% bound water by weight. The low partial pressure of CO in the Martian atmosphere probably would not pose serious problems to the organic synthesis since CO_2 , which is present in large quantity, also could react with H atoms to form CO_2H radicals. The effect of O_2 on these syntheses is minimum, since in our ex-

periment the addition of 1 mmHg of O_2 to the CO-CO_2 mixture adsorbed on silica gel showed little effect on the CO_2H radical yield (Fig. 1). We suggest that further study of such heterogeneous systems may provide a mechanistic basis for gauging the potential importance of gas-solid photochemistry for chemical evolution on other extraterrestrial bodies, on the primitive Earth, and on dust grains in the interstellar medium¹⁹.

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Microearthquake survey of Median Valley of the Mid-Atlantic ridge at $36^{\circ}30'\text{N}$

MICROEARTHQUAKE activity in the median valley of the Mid-Atlantic Ridge near $36^{\circ}30'\text{N}$ has been monitored using expendable radio-sonobuoy arrays. Similar techniques have been used by Reid *et al.*¹ in the Gulf of California, Macdonald and Mudie² near the Galapagos spreading centre and recently by Reid and Macdonald³ near fracture zones A and B shown in Fig. 1 (ref. 4). This study provides area coverage not obtained by Reid and Macdonald whose experiment was confined to the fracture zone regions. A new method of sonobuoy position determination was used that provides relative buoy locations (locations with respect to each other) to within ± 10 m. The ultimate positioning capability in latitude and longitude depends on the accuracy of the primary navigation system available. Satellite navigation can yield absolute positions to within ± 100 m. An estimated actual accuracy of ± 200 m is ascribed to the experiments

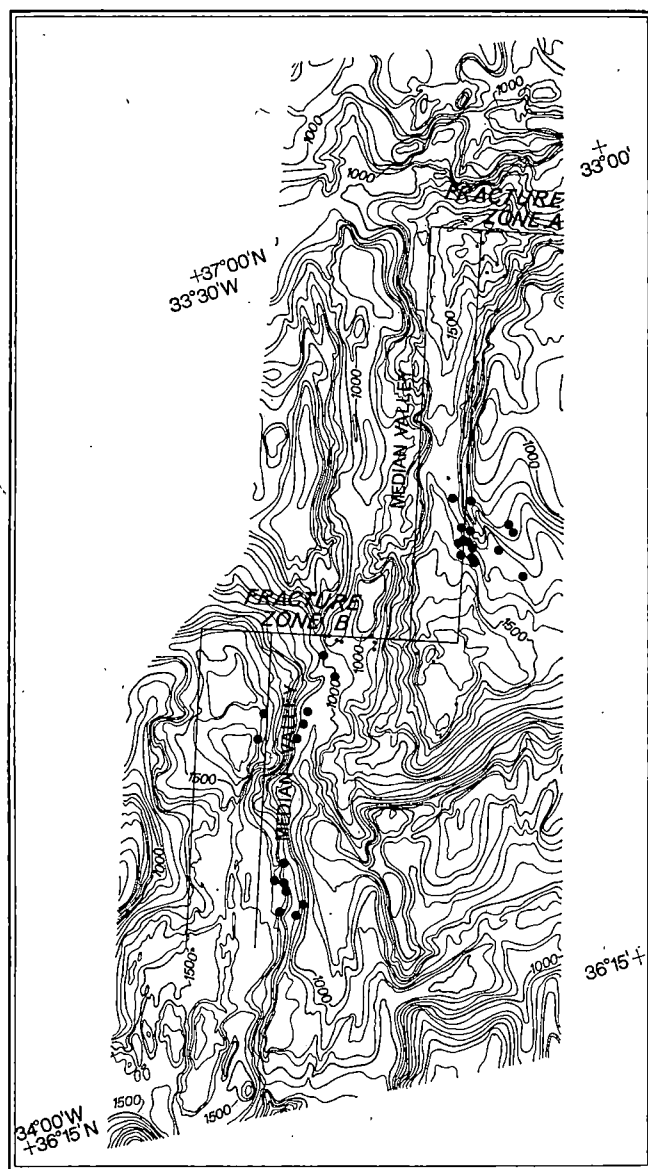


Fig. 1 Bathymetric map of part of the Mid-Atlantic Ridge. Epicentre locations obtained in this study are shown as large circles. Small circles represent epicentres determined by Reid and Macdonald³. Approximate location of fracture zones and median valley is shown. Contours are in uncorrected fathoms.

reported here. Accuracy of this order allows correlation of epicentre locations with topographic features of similar scale size. Recently acquired bathymetric data (K. C. Macdonald and colleagues, in preparation) indicate that significant features of the ridge near 36°30'N, such as transform faults and valley walls, can be defined by scale sizes of some hundreds of metres. A total of 112 events definitely attributable to seismic activity were recorded in 72 h of listening, yielding an average rate of occurrence of 1.5 events h^{-1} .

Arrays consisting of three US Navy AN/SSQ-57 calibrated sonobuoys, with 91.4-m hydrophone depth, were launched at three locations within the study area. The arrays approximated equilateral triangles with legs of 1.5 to 2 km. Sonobuoy positions were continuously monitored in real time by an acoustic navigation system originally designed to track the course of the submersible DSRV Alvin, but modified to track a moving hydrophone. The system uses either two or three bottom-moored acoustic transponders and a shipboard transmitter/receiver. (Two transponders make orientation with respect to the transponder baseline ambiguous; three transponders resolve the ambiguity. Often the ambiguity can be resolved from bathymetric data, or alternative navigation

aides, thus allowing the use of only two transponders.) Determination of the position of a sonobuoy consists of first measuring the round trip travel time of an acoustic pulse emitted by the ship, received and retransmitted at a different frequency by each transponder and received aboard ship. This observation provides the one way travel time from ship to transponder, t_1 . A second transmitted pulse is received by the transponders and retransmitted, but this time is received by the sonobuoy, thus determining the ship-transponder-sonobuoy acoustic travel time, t_2 . The travel time from transponders to buoy is $t_1 - t_2$. Slant ranges to the transponders are computed, corrections are made for sound velocity variation with depth (ray bending), and buoy position is resolved into a rectangular coordinate system whose origin is fixed with respect to the transponder net. Travel times can be determined to within 2 ms, thus establishing a limitation of about ± 3 m in buoy positioning. In practice a sonobuoy fix can be obtained every 20 to 30 s. (The maximum round-trip travel time range of the navigation system was about 12 s.) Absolute positioning of the transponder net in latitude and longitude is done using satellite fixes.

Seismic signals from the sonobuoy array elements were low pass filtered (0 to 500 Hz) and recorded on an f.m. analogue tape recorder with frequency response ± 0.7 dB from d.c. to 5 kHz. An example of three events received by a single sonobuoy within an interval of about 40 min is shown in Fig. 2. The event depicted in Fig. 2a occurred within the bounds of the triangular array, less than 1 km away from all sonobuoys. First compressional wave arrival (P) and two reflections of that wave through the water column (P_1 and P_2) are clearly shown. Shear waves (S) and direct water waves (T) are not evident because of the proximity of the event. Figures 2b and 2c are more distant and exhibit prominent T-phase arrivals. These figures give an indication of the excellent recording conditions encountered. Sea states were typically 0 to 1/2 with wind and swell near zero. Ambient noise levels at 25 Hz were chiefly less than -10 dB with respect to $1 \mu\text{bar}$. It is estimated that the array could detect microearthquakes of magnitude $M > -1$.

Power spectra of the events shown in Fig. 2 are shown in Fig. 3. These spectra have been corrected for the rising frequency response of the calibrated sonobuoys and represent an earthquake signal in excess of background noise. The spectra peak is about 15 to 25 Hz, rather greater than the typical peak frequencies of distant events⁵. This is not surprising since there is increased attenuation of high frequencies over long propagation paths. Measured peak pressures are

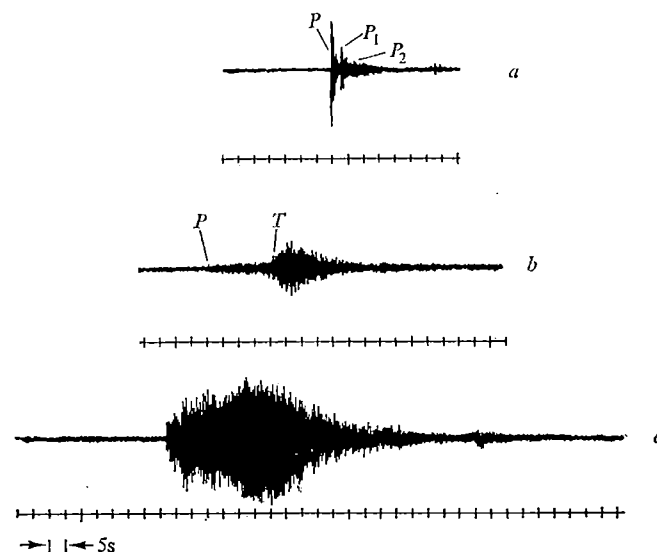


Fig. 2 Example of microearthquakes received with a single sonobuoy. Event a is less than 1 km horizontal range from the receiving hydrophone. a, 0231:30; b, 0156:40; c, 0154.

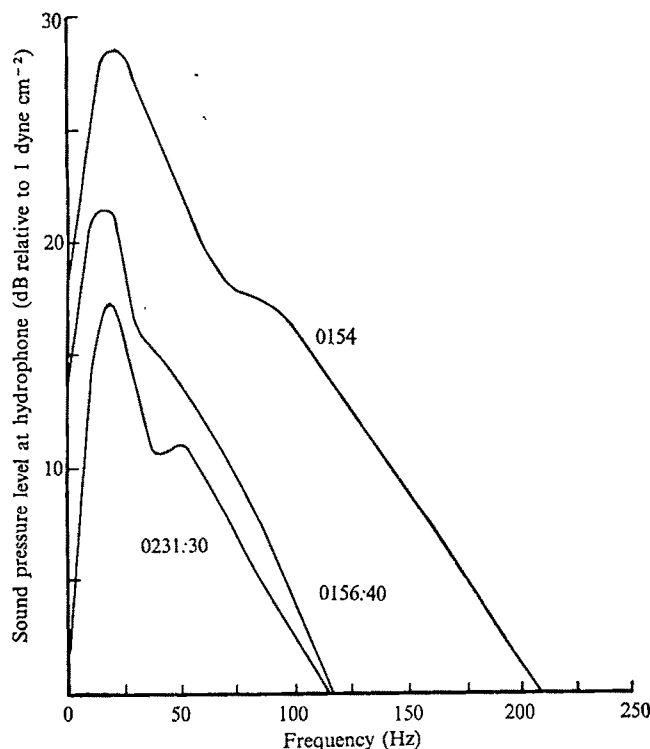


Fig. 3 Power spectra of three typical events (signal in excess of noise).

comparable to an equivalent source strength of some 75 to 100 dB relative to 1 μ bar at 1 m assuming spherical spreading over the entire transmission path.

Earthquake epicentre locations were determined by measuring the relative arrival times of the initial P wave on all three buoys. A horizontal, plane bottom was assumed, characterized by a compressional velocity of 5,000 ms^{-1} . When S-wave arrivals were distinct, S-P travel time differences were also used. The arrival times were solved iteratively for epicentre location using a computer. Geographical location accuracy is estimated to be within ± 200 m, the major sources of error being the estimate of absolute latitude and longitude of the transponder navigation net, the assumed crustal compressional velocity of 5,000 m s^{-1} and the assumption of zero focal depth^{3,6}.

Although 112 events were recorded, only 29 had sufficiently distinct arrival times to allow accurate epicentre location. The small array dimensions (restricted by the range of the acoustic navigation net) made it impossible to locate events more than about 20 km away from the array. Thus it is difficult to compare the level of seismicity in the median valley with that near transform faults because virtually all recording time was over median valley segments. Reid and Macdonald measured about 0.5 events h^{-1} on fracture zones A and B, while we measured 1.5 events h^{-1} , but their system was less sensitive because of poor weather conditions and higher ambient noise levels.

All but two of the located events occurred along the boundary between the median valley floor and the median valley wall. Near-bottom, bathymetric profiles in the vicinity of the median valley both north and south of fracture zone B show that the median valley walls are constructed predominantly of normal faults (K. C. Macdonald *et al.*, unpublished). Since the microearthquakes in this study cluster at the edges of the median valley and not along the axis, the tremors are probably associated with the incipient and ongoing uplift of fault blocks forming the wall rather than with the dynamics of intrusion in the centre of the median valley. Indeed, the events located occurred only along the eastern edge of the median valley where the east rift wall

begins, even though the array detection range was sufficient to detect events along the west wall and valley axis. This asymmetry is also apparent in the bathymetry and magnetics; the regional gradient of the rift walls is more gradual on the east side, and the axis of the central magnetic anomaly is offset several kilometres to the east (ref. 5 and H. D. Needham and F. Francheteau, to be published). Although our sampling is over a very short time interval, the seismicity distribution suggests that seafloor spreading activity here may be highly asymmetric, skewed towards the east at present.

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Velocity ratios for rocks with oriented microcracks

THE velocity of sound in 55 gneisses from the Alps has been determined by Johnson and Wenk¹ in ambient conditions. Because of their foliated fabric, these rocks show a strong anisotropy in physical properties chiefly on account of preferentially oriented microcracks at the boundaries of mineral grains. Johnson and Wenk's determinations were made along the principal fabric directions, assuming orthorhombic symmetry; following their notation, the *c* direction is perpendicular to the foliation and therefore to the oriented microcracks, and the *a* and *b* directions lie within the plane of foliation. Along each direction of wave propagation, there are two shear velocities, corresponding to the two mutually perpendicular directions of polarisation parallel to the principal axes.

We analysed the data provided by Johnson and Wenk and found that, at 95% confidence interval, the mean values of the ratios between the velocities of the compressional waves and shear waves are: along the *a* direction, $\alpha_a/\beta_{ab} = 1.42 \pm 0.02$ and $\alpha_a/\beta_{ao} = 1.65 \pm 0.03$; along the *b* direction, $\alpha_b/\beta_{ba} = 1.59 \pm 0.02$ and $\alpha_b/\beta_{bo} = 1.77 \pm 0.03$; and along the *c* direction, $\alpha_c/\beta_{ca} = 1.29 \pm 0.02$ and $\alpha_c/\beta_{cb} = 1.26 \pm 0.02$. Thus there is distinctive anisotropy in the velocity ratios for the *a* and *b* directions where the polarisation of shear waves is either perpendicular or parallel to the foliation. On the other hand, along the *c* direction, where the polarised shear waves all lie within the plane of foliation, there is little anisotropy in the velocity ratio. A more interesting result is that the ratios for the *c* direction are very much

lower than those along the other directions, apparently because of the oriented microcracks perpendicular to the direction of wave propagation. These observations are in qualitative agreement with the theoretical prediction by Anderson *et al.*².

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Surface energy calculations for muscovite

Muscovite, $\text{KAl}_2(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2$, has cleavage properties which make it suitable for studying phenomena which occur on surfaces and interfaces. The excellent cleavage produces surfaces which will readhere with the same or nearly the same strength¹⁻³, and which can be molecularly smooth over large areas⁴. Experimental results indicate that the attraction across the (001) cleavage is greatest in a vacuum and becomes less in air or various other media, and there is evidence that the rate of cleavage influences the strength of attraction⁵.

Theoretical studies have been based on the assumption that electrostatic forces provide the major component of the interlayer attraction and this is supported by the observation that cleaving mica creates electrical charges on the cleavage surfaces. Computations depend on a knowledge of the arrangement of the atoms on the surface of the cleavage plane, known ionic charges, and the distances to ions on the other side of the cleavage plane. Splitting occurs along the plane containing the potassium ions, which are equally distributed on both new surfaces. This has been confirmed using Auger electron techniques⁶.

Earlier calculations have two major defects. First, information about the crystal structure has been based on an idealised arrangement of atoms and is therefore inaccurate. Second, electrostatic energy has either been calculated for pairs of charges on each side of the cleavage, or else a two-dimensional summation has been used.

In fact, the structure of muscovite is very different from the ideal structure. There is a considerable distortion from the ideal hexagonal arrangement of oxygen ions and the potassium ions have six coordinating oxygens rather than twelve⁷. Accurate refinements have culminated in a neutron diffraction study which located the hydrogen ions⁸.

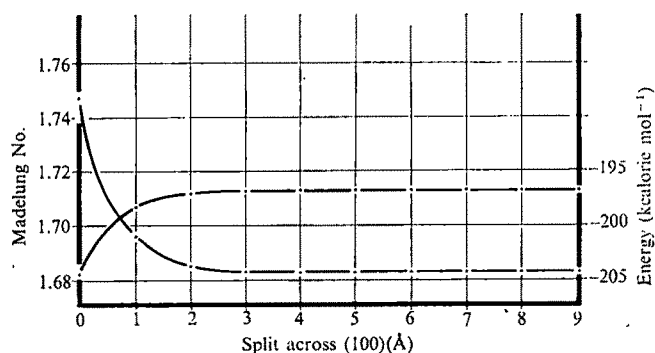


FIG. 1 A plot of the electrostatic energy (●, right axis) and Madelung number (▲, left axis) against the amount of split across the (100) face of NaCl.

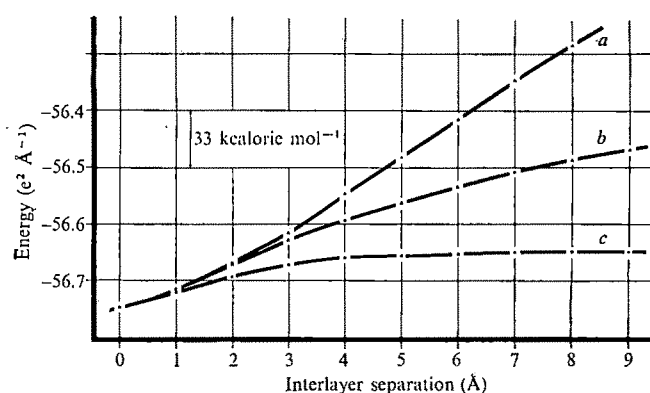


FIG. 2 A plot of electrostatic energy against the increase in the interlayer region between the aluminosilicate layers of muscovite. Curve *a* refers to the case in which the K ions remain in a plane equidistant from the two neighbouring mica layers; curve *b* is the case in which all the K ions remain on one layer and curve *c* represents an ordered arrangement with half of the K ions on each layer.

Electrostatic forces act over long distances and a correct calculation should take into account all of the atoms in the structure, and not just those on the cleavage surface. The surface energy represents the energy difference between a single crystal and one which has been separated into two parts across a plane. We have calculated the electrostatic energy of the normal crystal and of a series of structures which have an increased distance between atoms on either side of the plane. As the distance across the plane increases, the electrostatic energy approaches the energy for an infinite separation. These values give the surface energy directly.

As an example, the surface energy for the (100) face of NaCl has been calculated. The electrostatic energies of the normal and expanded structures were obtained using a computer program¹⁰. Figure 1 shows the Madelung number (left side) and the electrostatic energy (right side) against the increase in distance across the (100) face (horizontal axis). The Madelung number and electrostatic energy rapidly approach 1.68231 and $-196.87 \text{ kcalorie mol}^{-1}$ respectively, corresponding to a surface Madelung number of 0.06525 and a surface energy of $7.63 \text{ kcalorie mol}^{-1}$. These are in excellent agreement with earlier results¹¹.

The results of similar three dimensional calculations for the (001) plane of muscovite, based on the refinement of Rothbauer are shown in Fig. 2. Three distributions of interlayer potassium resulted in the three curves: curve *a* is for a structure in which the potassium ions remain in a plane equidistant from both neighboring layers; curve *b* is the case in which all of the potassium ions remain on one cleavage surface; curve *c* is for an ordered array of half of the potassium ions on each surface. Model *a* is clearly an unrealistic structure. This is evident from the energy plot which shows the greatest energy input required for cleavage. Model *b* is also energetically unfavourable but may represent the situation locally on a cleavage surface. Model *c* is the ordered array generated when the potassium ions at $z = \frac{1}{4}$ and $\frac{3}{4}$ are split so that two ions normally related by the c-face centring become crystallographically independent and remain on opposing surfaces. It is clear that for the ordered array, the electrostatic energy rapidly assumes a constant value which is the energy of an isolated mica layer. The energy difference between the normal and 'cleaved' mica is $0.098 \text{ e}^2 \text{ Å}^{-1}$ ($32 \text{ kcalorie mol}^{-1}$, 891 mJ m^{-2}).

The behaviour of the three models can be understood by considering the expression for the electrostatic energy:

$$\frac{-e^2}{2} \sum_{r=1}^{\infty} \sum_{s=1}^N \frac{Z_r Z_s}{R_{rs}}$$

where e is the charge on the electron, Z is the ionic charge

in one of the atoms in the structure, N is the number of atoms in the unit cell and R is the interionic distance. The arrangement of ions in model a is such that as the layers separate, the K^+-K^+ interactions remain constant while the K^+-O^- interactions decrease. The result is a decrease in the electrostatic energy. Model b is similar but there are fewer K^+-O^- interactions which become smaller. The K^+-O^- interactions in model c change as in model b , but many of the K^+-K^+ interactions decrease.

The surface energy of muscovite in a very high vacuum (1×10^{-13} torr) is about $5,000 \text{ mJ m}^{-2}$ ($168 \text{ kcal mol}^{-1}$, $0.05 \text{ e}^2 \text{ \AA}^{-1}$) (ref. 3). This large value implies that the potassium ions in real crystals are not ordered, and the energy-separation curve will lie between curves b and c (Fig. 2). In addition, if there is a high rate of splitting, the chances of maintaining locally ordered regions of potassium will be less than if the rate is low. A crystal which has been rapidly split would have an experimental curve closer to b than would a crystal split more slowly. This is in agreement with the observation that more energy is required to cleave mica rapidly⁵. It is not sufficient to assume that an average of half of the potassium will be on each cleavage surface; the arrangement of the potassium ions on the surface is an important factor in determining the interlayer attraction.

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New calcium silicate phase in hydrothermally treated γ -dicalcium silicate-quartz mixtures

HYDROTHERMALLY treated γ -dicalcium silicate-quartz mixtures have been investigated by several authors as a potential calcium silicate building product which, because of its low water content, could be used as refractory or heat insulating material. The phase composition of material formed at 150–600° C and 5–300 bar, for bulk molar Ca/Si ratios between 3.0 and 1.5 has been determined by Speakman *et al.*¹. Jernejčič *et al.*^{2,3} investigated lower Ca/Si molar ratios, that is, between 1.5 and 0.5 at 180–250° C, under saturated and superheated steam conditions. Superheated steam conditions during the initial period of hydrothermal reaction gave an unknown phase with a strongly pronounced diffraction maximum of 3.15 Å in the multiphase reaction products for bulk Ca/Si ratios lower than 1.25. Using previously recorded crystallographic data for calcium silicates and calcium silicate

hydrates, the maximum of about 3.15 Å could be assigned to the gyrolite group of hydrates. These phases, however, exhibit strong basal reflections at 22 and 19 Å. These basal reflection were not observed and identification was therefore necessary, particularly because there were no signs of pseudowollastonite in samples heated to 1000° C, as should have been the case if gyrolite or truscotite or a mixture of both were originally present.

To study the silicate structure of the 3.15 Å phase, the samples were treated with 0.1 M HCl and afterwards analysed by X-ray diffraction. The patterns obtained after acid treatment showed that the 3.15 Å phase remained even when all the coexisting xonotlite had been destroyed. This observation suggests that the silicate anions in the 3.15 Å phase are relatively highly condensed. Although the samples investigated varied in their starting Ca/Si ratio and in the original phase composition, all gave the same pattern after acid treatment. It was therefore obvious that the pattern belonged to one and the same phase. Table 1 gives this pattern.

To index reflections for the 3.15 Å phase the unit cell parameters and symmetry were found by selected area electron diffraction. Microscopic observation of samples rich in the 3.15 Å phase showed lath-like oriented aggregates. Near the zero setting of the goniometer most of the particles gave a characteristic electron diffraction pattern (Fig. 1). To ensure reliable measurements of values of d^* from this pattern the 2.338 Å powder ring of aluminum was used as internal standard; this gave 11.8 and $3.7 \text{ \AA} \pm 1\%$ for the two perpendicular repeat distances. The crystals are elongated parallel to the 3.7 Å direction, which was chosen as b . The repeat distances are similar to the parameters a and $b/2$ for tobermorite, but the general features of the tobermorite (001) electron diffraction pattern are quite different from that of the 3.15 Å phase. Whereas tobermorite shows marked pseudohalving of a and b , and only those reflections which $h + k = 2n$ (for the pseudocell), the 3.15 Å phase shows a primitive (001) reciprocal lattice section. The two phases also differ in c . The third row spacing of the reciprocal lattice for the 3.15 Å phase was derived from series of electron diffraction patterns obtained by tilting crystals about a ; this gave $c = 7.0 \text{ \AA}$. The electron diffraction patterns contained streaks that showed true b and c to be double those determined from the strong reflections only. The repeat distance of 7.4 Å in the b direction indicates that the silicate part of the structure consists of disordered dreierketten. The streaks of strong intensity, which were observed for $k + l = 2n$, indicate that the suffix $A \propto 22$ for the reciprocal lattice of the 3.15 Å phase. There are also, however, weak streaks for $k + l = 2n + 1$, so the true suffix should be $P \propto 22$ (ref 4). Electron diffraction patterns obtained by tilting

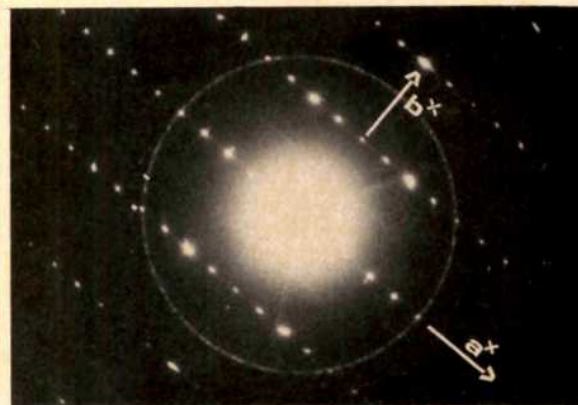


FIG. 1 Characteristic selected area electron diffraction pattern of 3.15 Å phase (corresponding to (001) reciprocal lattice section).

TABLE 1 X-ray powder data for 3.15 Å phase

d_{obs}	I/I_0	d_{cal}	h	k	l
6.99	11	7.000	0	0	2
3.971	20	3.968	3	0	0
3.660	25	3.678	3	0	2
3.230	47	3.238	1	0	4
3.210	47	3.215	1	2	2
3.150	100	3.148	2	2	0
2.805	57	2.796	2	2	2
2.710	16	2.710	3	2	0
2.610	5	2.612	3	2	2
		2.611	4	0	2
2.532	13	2.540	1	2	4
2.335	9	2.334	0	0	6
2.130	10	2.126	4	0	4
2.051	19	2.051	3	2	4
1.981	34	1.984	6	0	0
		1.982	6	0	2
1.852	32	1.855	0	4	0
		1.852	5	0	4

crystals about a^* and b^* also showed that the phase is monoclinic.

Using the unit cell parameters approximately determined from electron diffraction patterns it was possible to index all the reflections appearing on the X-ray diffraction pattern (Table 1). The unit cell parameters refined from the X-ray data are: $a = 12.02$ Å, $b = 7.42$ Å, $c = 14.14$ Å and $\beta = 98^\circ$. As already mentioned, the value of b indicates that silicate chains, similar to those existing in compounds of the wollastonite type are present in the 3.15 Å phase; consistent with this indication the 3.15 Å phase transforms into β -wollastonite at 700° C. The phase transition brought about by thermal treatment was followed on the sample richest in the 3.15 Å phase; it also contained unreacted quartz and a little xonotlite. On heating the sample to 680° C the X-ray pattern of the 3.15 Å phase did not change markedly. In a sample treated at 700° C, β -wollastonite and quartz were the only phases identified. The sample containing the highest proportion of the 3.15 Å phase was also used to evaluate approximately the Ca/Si molar ratio of the phase. Calculations using the weight fraction of unreacted quartz determined by X-ray diffraction and the loss on ignition gave a molar Ca/Si = 0.84. The ratio was also determined by electron microprobe, using a Philips EM300 electron microscope and an energy dispersive X-ray analysis system; after applying corrections⁵ an average molar ratio of 0.81 ± 0.03 was obtained. The amount of structurally important water in the 3.15 Å phase has not yet been precisely determined, but according to differential thermal analysis, thermal gravimetry and differential thermal gravimetry measurements it must be low (about 1%). Further work on the exact determination of the water content and the possible arrangement of atoms in the 3.15 Å phase is in progress.

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New method of nucleating diamonds

SYNTHESIS of diamond from graphite in the size range <0.1 to 1 mm requires an ultra-autoclave capable of operating at about 100,000 atm and 2000° K (ref. 1). A molten metal solvent-catalyst is also needed so that the conversion may be achieved in a relatively short time (minutes). Catalysts used include chromium, manganese and tantalum plus all elements of Group VIII of the periodic table, but nickel is used most frequently. The cross hatched region of Fig. 1 shows the combinations of pressure and temperature used for diamond synthesis when nickel is the catalyst.

It is advantageous to use metal-coated diamonds in resin-bonded wheels when grinding relatively ductile materials such as cemented tungsten carbides or hard tool steels. Nickel coated grains are most commonly used for wet grinding and about 55% of the weight of diamond is deposited as nickel which corresponds to a 15% increase in volume or an increase in diameter of about 15%. The metal coating is believed not only to provide a larger area for bonding but also promote heat transfer away from the wheel surface and to retain particles in the wheel face when grains fracture due to mechanical shock or thermal fatigue.

As well as these thermal and mechanical influences the metal coating could act to decrease wear by a chemical mechanism. At atmospheric pressure diamond represents an unstable form of carbon which will tend to transform to graphite if the temperature is sufficiently high and the soft transformed graphite will be rapidly worn away. A very thin coating of nickel will smear over the surface of an active coated diamond grain and in so doing could alter the

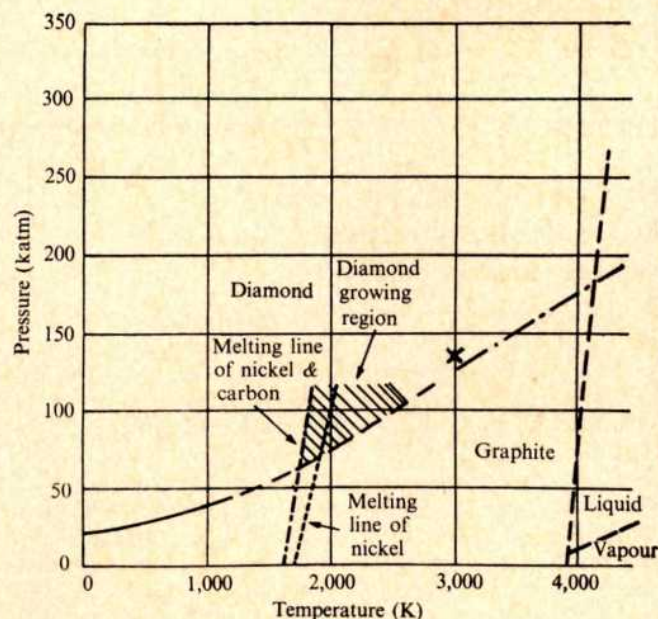


FIG. 1 Diamond-graphite equilibrium diagram with the diamond-growing region (using a nickel catalyst) shown cross hatched (ref. 2).

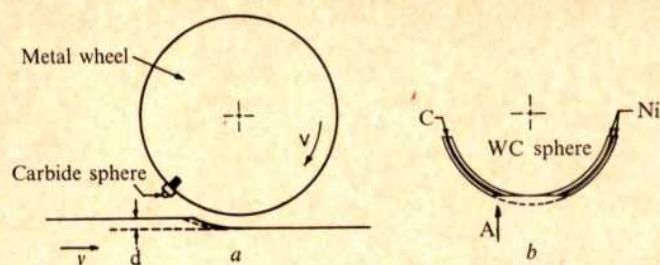


Fig. 2 a, Single-point cutting arrangement; b, tip of worn tungsten carbide sphere.

rate of wear by changing the equilibrium between the conversion of diamond to graphite and the conversion of graphite to carbon. Local conditions at the tip of the abrasive grain will be in the range of those found in the apparatus used to synthesize diamond from graphite in the presence of nickel.

When grinding steel, surface temperatures estimated as $\sim 3000^\circ\text{C}$ are obtained³ with grain-chip contact pressures as high as 2×10^6 pounds inch⁻² (130 katm)⁴. This condition marked by a cross on Fig. 1 is seen to exceed somewhat the range of operation of that used in diamond synthesis (cross hatched region). It is to be expected that under such extreme conditions the diamond nuclei produced will be extremely small, closely spaced and spontaneously formed.

The extreme conditions found at the tip of a cutting tool have been used previously to promote chemical reactions in organic synthesis in the process known as mechanical activation⁵. The very high temperatures and pressures existing at the tool tip constitute autoclave conditions without the need for an actual autoclave. This, together with the unique cleanliness of the nascent surfaces generated, causes an immediate reaction which is of great importance for organometallic reactions that are autocatalytic, such as those of the Grignard type.

To test the feasibility of generating diamond nuclei at the tip of an abrasive grain we conducted the following experiment. A tungsten carbide sphere, 1.5 mm in diameter, was coated with layers of nickel and graphite. The tungsten carbide sphere was first cleaned by sputter etching and then three thin layers were deposited in the order nickel-carbon-nickel using a sequential sputtering apparatus.

The coated tungsten carbide sphere was then cemented into a hollow head screw which in turn was threaded radially into the surface of an aluminum disk 8 inches (20 cm) in diameter. The disk was mounted on the spindle of a surface grinding machine in place of the usual grinding wheel and used to cut a groove in a piece of hardened ball bearing steel (AISI52100 steel of hardness R_c61) as shown schematically in Fig. 2. The speed of the disk (V) was in the range of conventional grinding (6,000 feet min⁻¹ or 30 m s^{-1}) while the table speed (v) and depth of cut (d) were adjusted to provide individual chips comparable in size to those in ordinary surface grinding ($v = 6\text{ inch min}^{-1}$ or 2.54 mm s^{-1} and $d = 0.001\text{ inches}$ or $25\text{ }\mu\text{m}$). The length of groove produced was 0.5 inches (12.5 mm) which corresponds to 250 individual cuts.

After cutting, a small flat was found on the tip of the tungsten carbide sphere (Fig. 2b). When the worn surface of the composite coating was examined in a scanning electron microscope for evidence of diamond nuclei from direction A in Fig. 2a, many small nucleation sites about $1\text{ }\mu\text{m}$ in diameter were evident at the inner nickel layer (Fig. 3a). The nuclei shown in Fig. 3a are believed to be diamond nuclei generated under the extreme conditions of temperature and pressure pertaining during cutting. Figure 3b shows a similar scanning electron micrograph of the composite surface abraded by hand on fine abrasive paper (4/0 silicon carbide) at low speed. In this case the abraded specimen is subjected to relatively low temperature, and hence conditions are not

favourable for the formation of diamond. The nuclei evident in Fig. 3a are not present in this case.

We used the Debye-Scherrer X-ray diffraction technique in an attempt to verify that the nuclei evident in Fig. 3a were in fact diamond. The sample was held stationary in the camera with the X-ray beam aimed at the wear scar. Tungsten carbide, titanium carbide and nickel patterns were clearly evident. In addition, one spot (corresponding to the strongest diamond line) was also found, possibly indicating the presence of diamond. The small concentration of nuclei makes it very difficult to obtain certain verification of their structure but work is continuing on this problem.

The practical implications of the possibility of generating diamond nuclei at the tip of an abrasive grain seem to lie in at least three areas:

- (1) The rate of wear of diamond grinding wheels could be reduced;
- (2) A source of diamond seeds for epitaxial growth of diamond by vapour phase deposition of diamond would be opened up;
- (3) It would provide a convenient method of studying the formation of new high temperature, high pressure stable phases of materials.

Some of the graphite formed by degradation of diamond during wear may be reconverted to diamond if nickel is present. The presence of nickel would thus alter the net rate of conversion of diamond to graphite. If so, it should be advantageous to provide a source of graphite to the diamond grain tip in addition to that coming from the conversion of diamond.

A diamond phonograph stylus might be made by coating an aluminum oxide cone of proper geometry with alternate layers of nickel and graphite. By making a few cuts in each of several directions seeds would be generated on the surface which could then be enlarged and subsequently grown together by epitaxial growth. The result would be a relatively large item of inexpensive material coated with a thin layer of diamond in the critical region only.

Although there is some evidence for the nucleation of diamonds by the technique reported here, it is not completely conclusive. But since the method is novel, simple and inexpensive, it could lead to many new important applications similar to those presented here. More important, if this

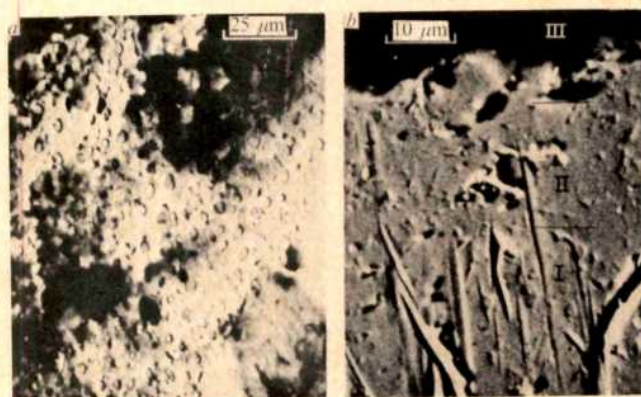


Fig. 3 a, Scanning electron micrograph of portion of inner nickel layer showing many (possibly diamond) nuclei; b, scanning electron micrograph of inner nickel layer abraded by hand at low speed. No nuclei seem to be present in this case. I, Tungsten carbide sphere; II, inner nickel layer; III, carbon layer.

mechanism is operating in grinding with diamond abrasive, it sheds light on the basic wear mechanism and suggests means for reducing the rate of wear in practice.

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Late Pleistocene desiccation along the White Nile

MORE than a century ago Baker¹ described the Blue Nile as a mountain stream, rising and falling with great rapidity, and the White Nile as a river of lake origin, flowing through vast treeless swamps in a land of "malaria, marshes, mosquitoes, misery". Three decades later Hull² noted that the flow of the main Nile had once been greater, and early this century a controversy arose which persists to this day. Was Nile discharge greater during the glacial periods of Europe and East Africa? Or were the glaciations coeval with tropical aridity?

Hume and Craig³ maintained that glacial ice caps in East Africa would displace the monsoon winds southwards, reducing precipitation over Ethiopia and thereby curtailing the flow of the Sobat, Blue Nile and Atbara, which currently account for more than 90% of the main Nile flood. Penck⁴ considered that when the northern Sahara was wet the southern Sahara was dry and encroached farther south. During a glacial period the arid belt lay 5° nearer the equator as a result of lower global temperatures and weakened atmospheric circulation.

More recently Fairbridge^{5,6} has equated Nile aggradation with diminished discharge during glacials, arguing that, since the Nile is now well able to transport its load to the delta, widespread silt deposition above the first cataract must imply reduced competence. Theoretical reconstructions of full glacial climates^{7,8} posit drier climates in areas as far afield as the south-west United States⁹, south-east Australia¹⁰, and parts of the Mediterranean littoral¹¹. In essence, lower temperatures imply lower evaporation rates⁷, which should result in lower rainfall^{5,7}.

Since 1960 many workers have reported evidence which seems to oppose the glacial aridity model and to favour the view that Nile discharge was greater than now during much of the late Pleistocene. Butzer and Hansen¹² referred to "revolutionary changes" in the late Pleistocene hydrographic regime of the Nile, and concluded that the wetter phases were synchronous in both Ethiopia and Egypt, except during deposition of the Masmas Formation at Kom Ombo about 20,000 yr BP. De Heinzelin^{13,14} also equated wadi activity in Nubia with higher rainfall in Ethiopia, Nile aggradation coinciding with conditions of maximum flow. Wendorf and Said¹⁵ described a phase of rising Nile level and widespread silt deposition in Nubia from 20,000 to 12,000 yr BP; and Wendorf and colleagues¹⁶ have distinguished three aggradational phases in Nubia between 16,000 and 12,000 yr BP, which were coeval with aridity in that region.

Three contradictory palaeoclimatic models purport to explain late Pleistocene aggradation by the main Nile. One equates aggradation with reduced Nile discharge and glacial aridity in Ethiopia^{5,6}. The second equates aridity with river incision, and greater Nile discharge with aggradation and wetter conditions in Nubia^{12,13}. The third equates siltation with high Nile levels and local aridity¹⁶. To resolve these issues the White Nile and the Blue Nile require separate study. We now present evidence which suggests that the White Nile had a much reduced and highly seasonal flow during the late Pleistocene before it rose to several metres above its modern unregulated flood level between 12,000 and 8,000 yr BP.

During a survey of prehistoric sites along the White Nile in January-March 1973 in association with J. D. Clark and an archaeological team from Berkeley, we collected microcrystalline dolomite and calcite from beneath subfossil freshwater shells which were laid down between 11,000 and 11,500 yr BP. When the shells were deposited, the White Nile flood level was 378 m, which is 5 m above the mean minimum and 2.5 m above the mean maximum water level¹⁹ at Esh Shawla before the Jebel Aulia dam was built (Fig. 1). The carbonate came from a depth of 3.75–3.90 m at site 2B and of 4.0–4.8 m at site 2. Above the carbonate was a layer of grey to yellow fluviatile sand from 0.5 to 1.1 m thick, locally with a basal admixture of clay and coarse quartz sand (Fig. 2).

An almost horizontal layer of greyish green lacustrine clay capped the sand and extended about 13 km west of the present White Nile to near Jebel et Tomat in latitude 13°36'N. The green clay was up to 1.5 m thick, with a surface elevation of 377.8 m in the west rising imperceptibly to 379.5 m some 7 km further east. Immediately above the green clay was a discontinuous shell-bed generally 5–10 cm thick made up of unbroken freshwater shells of *Cleopatra bulimoides* (Oliv.), *Melanoides tuberculata* (Müller) cf.

TABLE 1 Carbon-14 ages of shells in sediments bordering the White Nile

Site	Sample	Depth (m)	Elevation of base of shell beds (m)	Location	Age (yr BP)	Remarks
1	I 1486	1.45–1.70	377.3	13°35'N 32°41'E	11,300 ± 400	High river level ¹⁷
2	SUA-75	1.2–1.4	377.3	13°36'N 32°40'E	11,250 ± 220	High river level
3	I 1485	1.8–1.9	376.5	13°56'N 32°22'E	8,370 ± 350	High river level ¹⁷
3	SUA-68	1.2–1.4	377.0	13°56'N 32°32'E	8,130 ± 225	High river level
4	SUA-72	0.3–0.45	~398	15°23'N 32°22'E	8,400 ± 150	Local lakes full
4	SUA-74	0.45–0.60	~398	15°23'N 32°22'E	7,620 ± 130	Local lakes full
5	SUA-71	0.08–0.12	~401	15°22'N 32°21'E	7,870 ± 140	Local lakes full
6	SUA-73	0.12–0.20	~401	15°22'N 32°21'E	6,990 ± 100	Local lakes full
7	SUA-211	0–0.35	~378.5	13°34'N 32°34'E	5,500 ± 90	Pila midden on point-bar
8	SUA-67	0.4–0.8	384.2	13°36'N 32°44'E	4,540 ± 200	Top of dark cracking-clay
9	SUA-70	0.5–0.65	~377	13°56'N 32°21'E	3,030 ± 80	Pila midden on point-bar
10	SUA-212	Living		White Nile	150% modern	Living <i>Corbicula</i> shell
11	SUA-215B	Modern		White Nile	152.6% modern	Modern <i>Pila wernei</i> shell

Unless specified, all dates are from the Radiocarbon Laboratory, University of Sydney.

dautzenbergi Pilsbry and Bequaert, *Corbicula africana* (Krauss) and *Biomphalaria sudanica* (Martens). At site 2, fish spines and vertebrae were mixed among the shells, which gave a ^{14}C age of $11,350 \pm 220$ yr BP, in close agreement with the age of $11,300 \pm 400$ obtained from site 1^{17,20} (Table 1). Above the shell bed was a dark, cracking clay up to a metre or more thick, which accumulated in swamps left by the receding White Nile. Between about 12,000 and 8,000 yr BP, the White Nile remained high and formed an extensive lake up to 30 km or more wide (site 3, Table 1). Small lakes also existed west of the White Nile some 20 km north-west of Jebel Aulia (sites 4 to 6) between about 8,500 and 7,000 yr BP²¹, after which they remained quite dry.

Geochemical and palynological evidence indicate that between >14,500 and 12,000 yr BP Lake Victoria had no outlet²², the regional climate was dry, and dry forest grew on Ruwenzori²³. The White Nile was deprived of water from the Uganda lakes, so that its flow dwindled to a mere seasonal trickle. Dune formation was active along both banks of the White Nile between Kosti and El Geteina^{24,25} (Fig. 1) and deposits of very pure carbonate accumulated in or along the river, which contained abundant calcium and magnesium, indicative of the high salinity concentrations brought about by increasing evaporation downstream—a feature of modern rivers in arid areas²⁶. Site 2B contained both dolomite and calcite crystals, suggesting that when the dolomite was form-

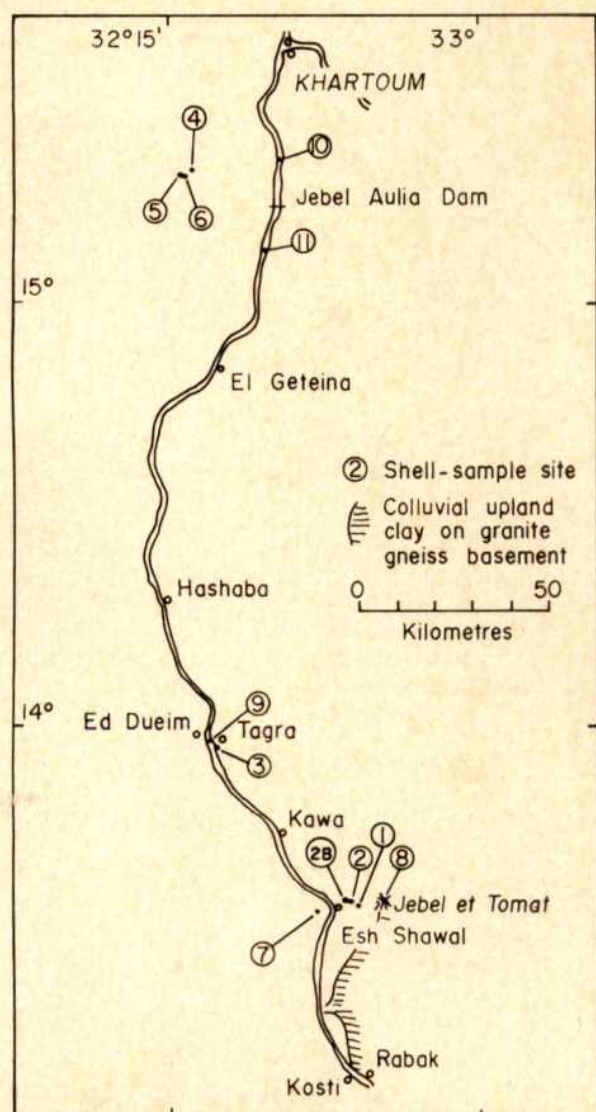


Fig. 1 Location of dated shell beds along the White Nile.

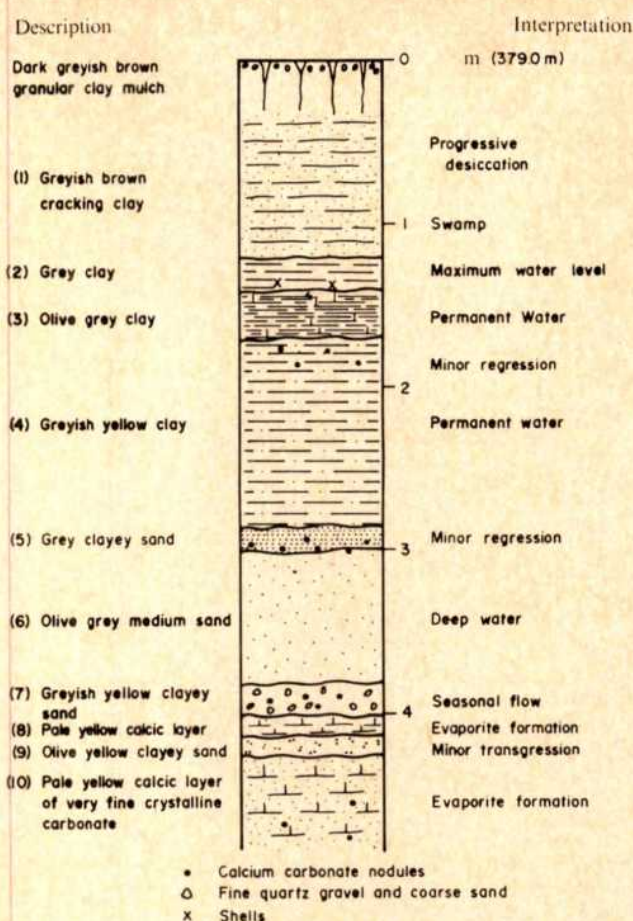


Fig. 2 Esh Shawal, section 2B.

ing magnesium-rich calcite was already abundant, and the ratio of dissolved Mg to Ca was at least 7 (ref. 27). It is worth noting that the levels of Lake Chad²⁸, Lake Rudol²⁹ and Lake Nakuru³⁰ were also very low at this time, and dunes were active in now vegetated parts of Nigeria³¹.

About 12,000 yr BP Lake Victoria overflowed, the level of the White Nile rose, and the dunes between Kosti and Ed Dueim became partly buried by alluvial clay. After 8,000 yr BP the level of the White Nile fell, dark clays accumulated in the swamps bordering the river until about 4,000 yr BP (Table 1, site 8), after which Neolithic man and increasing climatic desiccation accelerated the trend towards semi-desert conditions. The fixed dunes of Kordofan and the even flow of the modern White Nile indicate that the late Pleistocene dry period was more arid than the present semi-arid climate of central Sudan, and it is possible that the White Nile may have ceased to flow into the main Nile, which would thus have been even more seasonal in its regime than now.

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BIOLOGICAL SCIENCES

Non-specific 'pairing' of DNA molecules by recombination enzyme of *Bacillus subtilis*

ATP-dependent deoxyribonuclease(s) (ATP-DNase) is involved in the genetic recombination process in *Escherichia coli*¹⁻³, *Diplococcus pneumoniae*⁴ and *Bacillus subtilis*⁵ and may also play an important part in DNA repair⁶⁻⁸, DNA replication⁹ and cell growth¹⁰. ATP-dependent or ATP-stimulated DNases have also been isolated and characterised in other microorganisms¹¹⁻¹⁴. We reported the purification and properties of this enzyme in *B. subtilis*^{15,16} and noted that at the 600-fold purification level, the enzyme requires ATP for the hydrolysis of double stranded (ds) DNA, but not for single stranded (ss) DNA¹⁶. Since the activities on ds and ssDNA appear to reside in the same enzyme, we suggested the possibility that ATP consumption is somehow linked to the active unwinding of dsDNA before the DNase can hydrolyse phosphodiester bonds¹⁶. Recently, Friedman and Smith²⁴ have also proposed an active role of ATP in dsDNA unwinding by *Haemophilus influenzae* enzyme, which is based on the structural feature of intermediate product DNA. To examine this possibility further, we tried to visual-



FIG. 1 Electron microscopic study of ATP-DNase-DNA complex: control. The reaction mixture (50 μ l) with 0.5 mM ATP was incubated at 0° C for 2 min and at 32° C for 3 min without enzyme. $\times 37,254$.

ize the unwound portion of DNA and the DNA-enzyme complex by electron microscopy. Although the visual evidence is still ambiguous, an unexpected observation has been made. We report here the unusual property of *B. subtilis* ATP-DNase which apparently brings DNA molecules into close proximity or causes non-specific 'pairing' of dsDNA molecules.

ATP-dependent DNase was purified from *B. subtilis* sonicate as reported previously¹⁶. The sonicate of cells was centrifuged at 40,000g for 20 min. The supernatant was further centrifuged at 100,000g for 90 min, followed by streptomycin sulphate and ammonium sulphate fractionations. The fraction between 40 and 60% saturation of ammonium sulphate was further fractionated by agarose column (A 5m, Bio-Rad Laboratories) and DEAE-cellulose column chromatography. The DEAE-cellulose purified enzyme (600-fold purified) will be called here Fraction VI enzyme in conformity with the previous paper¹⁶. This enzyme was purified

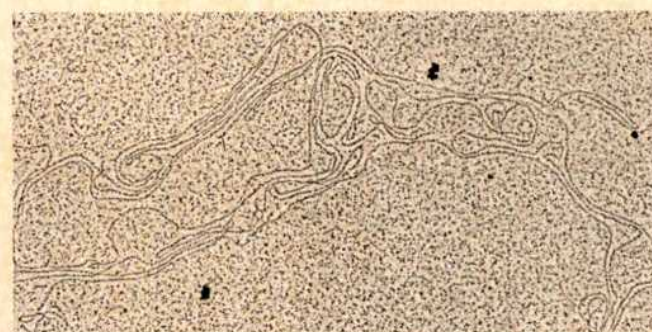


FIG. 2 Electron microscopic study of ATP-DNase-DNA complex. The reaction mixture (50 μ l) with ATP (0.5 mM) and one unit of Fraction VI enzyme (5.2 μ g protein). Incubation was for 2 min at 32° C. $\times 52,500$.

by glycerol gradient centrifugation to a final purity of approximately 5,000-fold. This latter enzyme preparation is called Fraction VIII.

The activity of ATP-DNase was measured by the release of acid-soluble DNA fragments from tritiated *B. subtilis* DNA. The DNA was phenol-purified according to the procedure of Saito and Miura¹⁷. Unless otherwise noted, the 'standard reaction mixture' (0.5 ml) contained the following: 25 mM Tris-maleate buffer (pH 7.5), 7 mM MgCl₂, 6 mM 2-mercaptoethanol, 0.5 mM ATP, 30 μ M nucleotide equivalent tritiated DNA, and 1–5 units of enzyme. One unit of enzyme activity hydrolyses 1 nmol of DNA nucleotide during 30 min incubation at 32° C.

The electron microscopic study of ATP-DNase on dsDNA was carried out as follows. Unless otherwise indicated, reaction mixtures contained the same components as the standard reaction mixture in the same concentrations except that the total volume was reduced to 50 μ l and 0.14 μ g of non-labelled phage λ DNA was used. Routinely a reaction was terminated by adding glutaraldehyde to the concentration of 10 mM at 0° C. The sample was kept for at least 10 min at 0° C and an aliquot was diluted with 0.01 M Tris-HCl, 0.001 M EDTA buffer, pH 9.0, to give 0.5 to 1 μ g ml⁻¹ DNA. Formamide and cytochrome *c* were added to final concentrations of 50% (v/v) and 0.01%, respectively. The sample was mixed thoroughly, and a few drops were spread on a water surface. DNA was picked up on carbon coated formvar grids. The sample was stained for 30 s in 0.001% solution of uranyl chloride in ethanol, washed briefly in 100% ethanol and air dried. Samples were shadow-casted with 80% Pt:20% Pd in a Denton DV52 vacuum evaporator and observed in a Phillips 300 electron microscope.

Phage λ DNA molecules incubated without enzyme in the presence of ATP and glutaraldehyde (control) were well spread and the majority of the molecules could be traced from



Fig. 3 Electron microscopic study of ATP-DNase-DNA complex. The reaction mixture (50 μ l) with ATP (0.5 mM) and 5 units of Fraction VI enzyme. Incubation was as in Fig. 2. $\times 60,000$.

end to end (Fig. 1). The control in the absence of ATP and glutaraldehyde showed similar pictures. When ATP-DNase of Fraction VI was present in the reaction mixture, however,

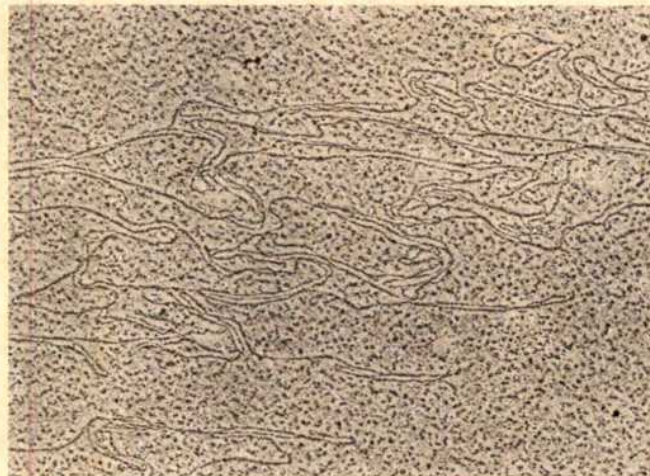


Fig. 4 Electron microscopic study of ATP-DNase-DNA complex. The reaction mixture (50 μ l) with ATP (0.5 mM) and one unit of Fraction VIII enzyme (0.7 μ g protein). Samples with one unit of enzyme purified on a glycerol gradient (Fraction VIII) were prepared and examined according to the procedure described in the text. $\times 64,220$.

a large portion of each DNA molecule was closely lined or 'paired' (non-sequence-specific pairing) (Fig. 2). Similar 'pairing' was also observed when ATP was withheld from the reaction mixture. Thus, omission of ATP from the reaction mixture in the presence of the enzyme did not significantly change the degree of 'pairing'. The degree of non-specific pairing was clearly dependent on the enzyme concentration in the reaction mixture (compare Figs. 2 and 3). In separate experiments, we have examined samples incubated with serially diluted enzyme (Fractions VI and VIII) in the electron microscope. The results showed that as the concentration of enzyme was reduced, the degree of 'pairing' also decreased. To try to eliminate the possibility that the non-specific pairing of DNA observed may be caused by some other contaminating proteins in the preparation, similar experiments to those described above using 600-fold purified enzyme (Fraction VI) were carried out using the same number of activity units of the 5000-fold purified enzyme (Fraction VIII). Figure 4 shows essentially the same degree of 'pairing' as that obtained using Fraction VI. In this case, also, omission of ATP gave a similar degree of 'pairing'.

Since the molecular weight of ATP-DNase of *B. subtilis* is estimated by sedimentation to be approximately 260,000, and since the enzyme purified on the glycerol gradient is fairly homogeneous (Ohi, Donovan and Sueoka, unpublished data), the reaction mixture of Fig. 4 contains approximately 1.5×10^{12} enzyme molecules and 2.5×10^9 molecules of λ DNA. If we assume that all enzyme molecules bind to DNA under this condition and also bind at random (for example, in a non-cooperative fashion) the average distance between enzymes would be 80 base pairs.

Other proteins, however, might cause similar non-specific pairing of DNA molecules as a result of cross-linking caused by glutaraldehyde fixation. Under our experimental conditions, addition of lysozyme to the reaction mixture in concentrations up to 500 μ g ml⁻¹ in place of ATP-DNase did not promote the non-specific pairing of phage λ DNA. In this respect, it should be noted that the reaction mixture contained 104 μ g ml⁻¹ (Fig. 2) and 520 μ g ml⁻¹ (Fig. 3) protein with Fraction VI enzyme and 14 μ g ml⁻¹ protein with Fraction VIII. Furthermore, various proteins have been reported not to cause such an effect on DNA: T4 gene 32 protein,

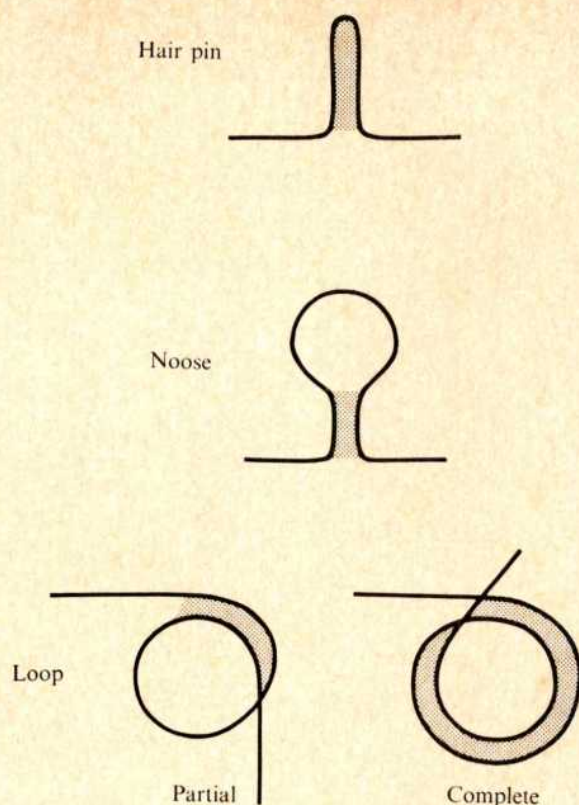


FIG. 5 Three basic configurations of dsDNA to dsDNA 'pairing'. The solid line represents double-helical DNA and the shaded area indicates pairing. The various configurations in Fig. 3 can be explained by combinations of these basic configurations.

RNA polymerase²⁰, cytochrome *c*, trypsin, chymotrypsin, dipropylfluorophosphate-treated trypsin and chymotrypsin, RNase, methylated-albumin, and lysozyme²¹.

Winder and Sastry²² reported that ATP-DNase of *Micrococcus smegmatis* formed a long-lived enzyme-DNA complex which could be trapped on the Millipore filter and assayed for its activity. Shemyakin *et al.*²³ also demonstrated the formation of the *B. subtilis* enzyme-DNA complex by glycerol gradient centrifugation. In separate experiments we have confirmed the existence of the enzyme-DNA complex by the Millipore filter method. These observations indicate that the ATP-DNase can form a stable complex with DNA. There are two possible modes of binding of the enzyme to DNA: one which enables the DNase to work in unwinding and hydrolysis, and one which causes the non-specific 'pairing' of DNA molecules.

From the present study, the detailed structure of the 'pairing' cannot be specified. The distance between the paired dsDNAs cannot be defined. DNA in Fig. 3 (high enzyme concentration) shows tight pairing, while DNA in other figures with 1/5 enzyme shows rather loose pairing. The difference may come from a higher resistance in the former preparation to stretching during the spreading with cytochrome *c*.

There has been no evidence of strand opening in any of the preparations we have looked at so far. This is not surprising since any strand separation should quickly be followed by annealing. All features of pairing in Fig. 3 can be accounted for by the three basic configurations schematically shown in Fig. 5 without introducing strand opening of double-stranded DNA. It is also clear that the 'pairing' is not limited to two dsDNAs. The sticking of more than two dsDNAs is seen in Fig. 3.

In conclusion, we have found that the ATP-DNase of *B. subtilis* brings DNA molecules into close proximity. We do not know at present the functional meaning of this 'pairing' nor its physicochemical basis. Since the enzyme is involved

in genetic recombination, however this property of the enzyme may have some significance in the mechanism of recombination and some other types of DNA-DNA interactions *in vivo*.

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Zinc ions inhibit replication of rhinoviruses

THE rhinoviruses comprise a subgroup of the picornaviruses, distinguished by their acid lability. They are medically important as the infectious agents of the common cold in man. Although there have been reports of chemical agents which inhibit rhinovirus replication, in only a few instances has the mode of antiviral action been demonstrated at a biochemical level^{1,2}.

Although metal ions are well known cofactors in many biological processes, and are inhibitory to some enzymatic reactions^{3,4}, they have received scant attention in virology. We have now examined the abilities of the following metal ions to affect the replication of twelve different picornaviruses in HeLa cells: cadmium, calcium, cobalt, copper, magnesium, manganese, mercury, molybdenum, nickel and zinc. They were supplied as the chloride or acetate, and were tested at 1, 0.1 and 0.01 mM. We used an agar overlay assay described before⁵, but omitted DEAE-dextran. The metals were in-

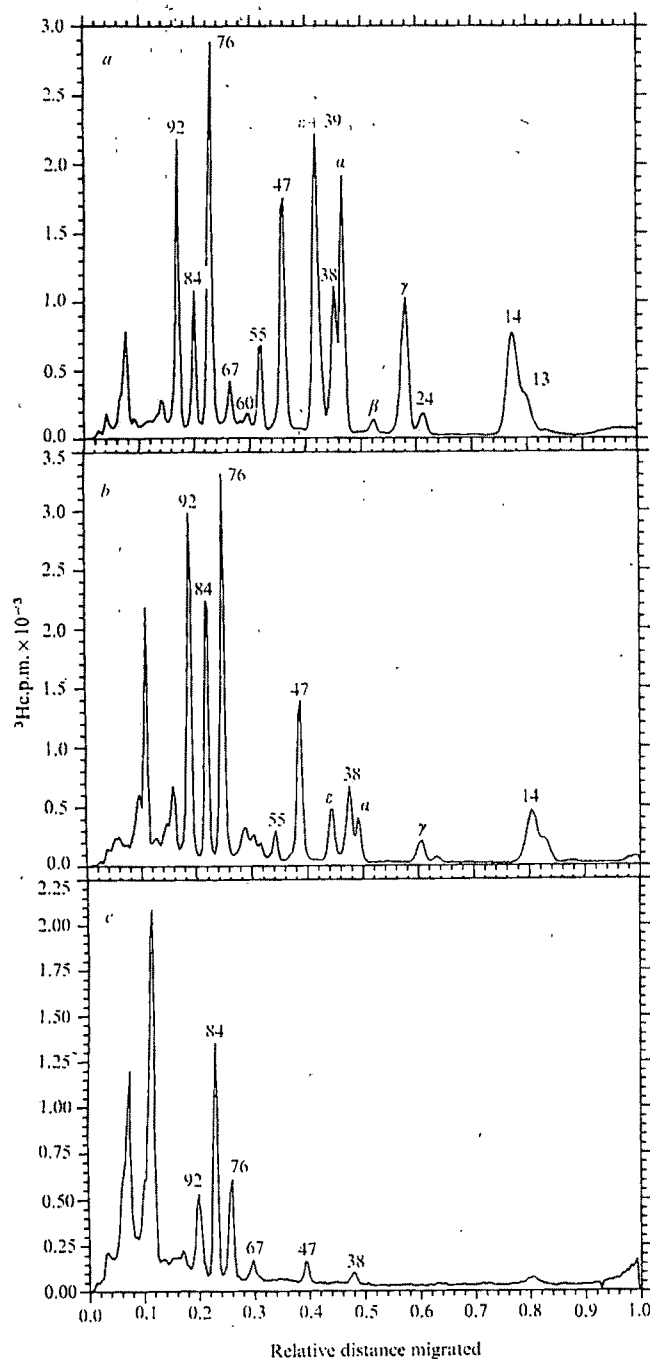


FIG. 1 Inhibition of cleavages of HRV-1A polypeptides by zinc. A suspension culture of HeLa cells (4×10^6 cells ml^{-1}) was infected with 100 PFU per cell of HRV-1A in the presence of $5 \mu\text{g ml}^{-1}$ of actinomycin D at 34°C . By 4 h after infection host protein synthesis was almost completely inhibited. Identical samples were labelled 4–5 h after infection with $50 \mu\text{Ci ml}^{-1}$ of a ^3H -amino acid mixture in the absence of added zinc (a), in the presence of 0.1 mM ZnCl_2 (b), and in the presence of 0.8 mM ZnCl_2 (c). The whole cells were then solubilised with 1% sodium dodecyl sulphate (SDS), dialysed, and subjected to electrophoresis on $0.6 \times 27 \text{ cm}$ SDS-polyacrylamide gels⁸. Migration was from left to right. The gels were then fractionated and radioactivity was measured with a liquid scintillation spectrometer. The data were analysed using a PDP-10 computer (Digital Equipment Corp.) and the electropherograms were plotted using a CalComp plotter. The gel patterns are photographs of the actual computer output.

incorporated into the agar and added to the HeLa cell monolayers after the virus had been attached to the cells for 1 h. Activity of a particular metal on a virus type was scored as the percentage reduction of the number of virus plaques in the presence of the metal compared with untreated cell

cultures. Toxicity to the cells was not measured quantitatively, but estimated by the loss of stained cells from the plastic Petri dishes after 4 d of incubation in the presence of a given concentration of the metal. If the cells displayed a toxic response, the antiviral activity of the metal at that concentration was not counted.

Only zinc displayed antiviral activity in non-toxic concentrations. Table 1 shows that eight out of nine rhinoviruses were sensitive to 0.1 mM zinc. There was a spectrum of sensitivities, with type 1A being most and type 14 least inhibited. One human rhinovirus, type 5, and two types of poliovirus were insensitive.

To determine the kinetics of zinc inhibition, HeLa cell monolayers infected with HRV-1A were exposed to 0.1 mM zinc at various times during a single replication cycle and the final yield of virus was determined in each case. The results indicated that the addition of zinc at any time during viral replication immediately inhibited further formation of infectious virions.

Two easily assayed processes of picornavirus replication are RNA and protein synthesis. Measurements of the effect of 0.1 mM zinc on total incorporation of ^3H -uridine, or amino acids into HRV-1A macromolecules, indicated that viral RNA and protein synthesis were reduced only by about 10–20%. This slight reduction was not sufficient to explain the marked inhibitions seen in plaque tests (Table 1).

The viral RNA acts as a message in the infected cell to direct the synthesis of the viral polypeptides. Some of the large polypeptides generated during translation are precursor molecules which undergo proteolytic cleavages to produce the smaller viral polypeptides^{6–9}. The most dramatic effect of zinc was to inhibit some of these cleavages of the HRV-1A polypeptides. Figure 1a shows the normal pattern of labelled viral polypeptides formed during 1 h of labelling. Addition of 0.1 mM zinc with the radioactive amino acids prevents post-translational cleavages and causes the accumulation of a set of large precursor polypeptides (Fig. 1b). We found that different cleavages were sensitive to different concentrations of zinc, and that progressively larger polypeptides could be accumulated by increasing the zinc concentration (Fig. 1b and c). Zinc also prevented the cleavage of HRV-2 precursor polypeptides in HeLa cells.

It is significant that the two types of poliovirus which could produce plaques in the presence of 0.1 mM zinc (Table 1) demonstrated normal intracellular polypeptide patterns (not shown) at a zinc concentration which markedly blocked cleavage of HRV-1A polypeptides. In suspension cultures and at higher concentrations of zinc, however, there is marked inhibition of cleavage of certain polio- and EMC viral polypeptides (B.E.B. and B.D.K., in preparation). The cleavage reaction most sensitive to zinc inhibition is the conversion of the capsid precursor, polypeptide 92, (Fig. 1b) to ϵ , γ , and α , the HRV-1A capsid polypeptides^{6–9}. Although other explanations are possible, we suggest that zinc interacts with the HRV-1A capsid proteins (B.D.K. and B.E.B., in

TABLE 1 Plaque-forming ability of picornaviruses on HeLa cells in the presence of 0.1 mM zinc chloride

Virus	% Reduction in No. of plaques
Human rhinovirus (HRV) 1A	99.99
HRV-1B	99.9
HRV-2	97
HRV-3	99
HRV-5	<10
HRV-14	85
HRV-15	99
HRV-39	99
HRV-51	90
Equine rhinovirus (Plummer strain)	95
Poliovirus type 1 (Mahoney)	0
Poliovirus type 2 (P712ch2ab)	0

preparation), blocking conversion of the precursor and rapidly preventing maturation of viral RNA and capsid polypeptides.

This report represents a direct demonstration that a chemical which blocks cleavage of viral protein precursors can act (in tissue culture) as an antiviral agent. Recent evidence for polypeptide cleavages during the replication of bacteriophages¹⁰ and many animal viruses⁹, including RNA tumour viruses¹¹, together with the results presented here, suggest that protease inhibitors¹²⁻¹⁴, or zinc may be useful in blocking certain stages of replication of many kinds of viruses.

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Genetic evidence for SV40 gene function in enhancement of replication of human adenovirus in simian cells

HUMAN adenoviruses undergo a complete cycle of replication in human cells, but cause an abortive infection in simian cells. In the simian cells infected with the human adenovirus, early adenovirus-specific T antigen is induced^{1,2}, viral DNA is replicated to the same level as in productive cycles of replication^{3,4}, and no change in the synthesis of viral mRNA can be detected^{5,6}. However, only limited amounts of viral capsid proteins are made in relatively few cells^{1,2,6-8}. If the simian cells are coinfecting with simian virus 40 (SV40), the nonproductive cycle of replication of human adenoviruses is changed to a productive cycle^{9,10}. This helper effect of infection by SV40 is thought to be under the control of SV40 gene function, but there has been no direct evidence for this. Using temperature-sensitive (*ts*) mutants of SV40, I have now found such evidence.

The isolation and characterisation of (*ts*) mutants of SV40 have been described already^{11,12} and the properties of the mutants are summarised in Table 1. In simian cells,

TABLE 1 Properties of the SV40 temperature-sensitive mutants

Complementation groups	I	II	III
Mutant	<i>ts</i> 662	<i>ts</i> 663	<i>ts</i> 640
Adsorption and uncoating	+	+	+
Synthesis of T antigen	+	+	ND
Synthesis of viral DNA	+	+	—
Synthesis of virion antigen	+	—	—*
Infectious virus	—	—	—
Morphological transformation			
Initiation	+	+	—
Maintenance	+	+	+
Heat stability of virus particles produced at permissive temperature	Reduced	Reduced	ND
Helper function for replication of human adenovirus in simian cells†	+	+	—

Data are taken from the results of the previous studies of Kimura and Dulbecco^{11,12}, unless otherwise specified.

+, Normal at nonpermissive temperature (39°–40° C); —, restricted at non-permissive temperature; ND, not determined.

* My unpublished observations.

† This study.

these mutants replicate well at the permissive temperature of 33° C but not, if at all, at the nonpermissive temperature of 40° C, while wild type parent viruses can replicate equally well at 33–40° C. Genetic analyses have so far revealed three cistrons in SV40 genome and representative mutants of each of three complementation groups (groups I–III) were used in the study described here (Table 1). Human adenovirus type 2 (Ad2) (supplied by Dr K. Fujinaga, Aichi Cancer Centre, Nagoya) was used because it can replicate relatively well at 33°–40° C and because it can be assayed relatively easily in the indicator cells used (fi-8 cells). The stocks of Ad2 were prepared in secondary or tertiary cultures of human embryonic kidney (HEK) cells after the virus had been plaque-cloned twice in HEK cells. The stocks of SV40 and its *ts* mutants were prepared in BSC-1 cells (subline P66) as previously described¹¹. Cultures of singly or doubly infected African green monkey kidney (AGMK) cells were collected for determination of infectivity 42 h (40° C) and 88 h (33° C) after inoculation. The yield of virus in these conditions represents a single cycle of virus development for both SV40 (ref. 11 and 12 and my unpublished observations) and Ad2 (ref. 13 and my unpublished observations).

Table 2 (experiment 1) lists the yield of Ad2, parental SV40, and *ts* mutants of SV40 of complementation groups I and II in the singly or doubly infected cultures of the CV-1 line of AGMK cells at 33° C and 40° C. Clearly the *ts* mutants of SV40 of groups I and II can enhance the replication of Ad2 at 33°–40° C with efficiencies comparable with those in parental SV40-infected cells at 33°–40° C. Essentially similar results were obtained using tertiary cultures of AGMK (Table 2, experiment 3). As it has been suggested that the genes identified in these mutants (cistrons I and II) probably code for SV40 virion proteins¹¹, my results indicate that the function of the SV40 virion proteins specified by these two cistrons is not necessary for the replication of human adenovirus in simian cells. Jerkofsky and Rapp¹⁴ have also shown that other SV40 *ts* mutants, which are probably in the same group as my group I, can help the adenovirus replication in simian cells at the nonpermissive temperature. In confirmation of the previous observations^{7,15}, infection by Ad2 in turn interferes to a great extent with the replication of SV40 in the simian cells (Table 2, experiment 1).

The results of mixed infections with Ad2 and an SV40 *ts* mutant (*ts*640) of complementation group III are shown in Table 2 (experiments 2 and 3). Clearly this mutant fails to enhance the replication of Ad2 at 40° C but not at 33° C both in tertiary cultures of AGMK and in cultures

TABLE 2 Replication of adenovirus type 2 in various AGMK cells coinfecting with SV40 or *ts* mutants of SV40

Experiment No. Cells	Virus	Input multiplicities (p.f.u. per cell)	Ad2 titre (p.f.u. ml ⁻¹)			SV40 titre (p.f.u. ml ⁻¹)		
			Background (33°C 15h)	33°C 88h	40°C 42h	Background (33°C 15h)	33°C 88h	40°C 42h
(1) CV-1 line of AGMK	Ad2	8	1.5 × 10 ¹	7.2 × 10 ²	2.0 × 10 ³			
	SV40	10				1.0 × 10 ⁵	1.5 × 10 ⁸	1.0 × 10 ⁷
	Ad2 + SV40	4 + 5		2.0 × 10 ⁶	3.9 × 10 ⁸		1.4 × 10 ⁸	5.5 × 10 ⁸
	<i>ts</i> 662 (group I)	37					1.6 × 10 ⁸	1.7 × 10 ⁸
	Ad2 + <i>ts</i> 662	4 + 18		4.8 × 10 ⁶	1.0 × 10 ⁶		3.7 × 10 ⁶	1.1 × 10 ⁶
	<i>ts</i> 663 (group II)	45					1.2 × 10 ⁸	2.1 × 10 ⁸
(2) Tertiary cultures of AGMK	Ad2 + <i>ts</i> 663	4 + 22		5.0 × 10 ⁶	2.3 × 10 ⁶		1.2 × 10 ⁶	5.1 × 10 ⁴
	Ad2	3	9.5 × 10 ²	1.2 × 10 ³	4.3 × 10 ³			
	SV40	4					5.8 × 10 ⁵	1.1 × 10 ⁶
	Ad2 + SV40	3 + 4	2.3 × 10 ³	3.1 × 10 ⁷	1.6 × 10 ⁶	1.7 × 10 ³	3.3 × 10 ⁶	3.2 × 10 ²
	<i>ts</i> 640 (group III)	8						
	Ad2 + <i>ts</i> 640	3 + 8	3.0 × 10 ²	8.0 × 10 ⁷	1.1 × 10 ⁴			
BSC-1 line of AGMK	Ad2	3	9.5 × 10 ²	8.0 × 10 ³	1.3 × 10 ⁴			
	SV40	4					3.8 × 10 ⁶	2.8 × 10 ⁶
	Ad2 + SV40	3 + 4		5.6 × 10 ⁷	1.6 × 10 ⁷			
	<i>ts</i> 640 (III)	8				1.2 × 10 ³	1.9 × 10 ⁷	7.8 × 10 ⁴
	Ad2 + <i>ts</i> 640	3 + 8		6.0 × 10 ⁷	9.3 × 10 ⁴			
(3) Tertiary cultures of AGMK	Ad2	9	1.4 × 10 ²	1.7 × 10 ²	1.5 × 10 ²			
	SV40	4				3.0 × 10 ¹	1.6 × 10 ⁵	2.8 × 10 ⁶
	Ad2 + SV40	9 + 4		5.5 × 10 ⁶	1.7 × 10 ⁵			
	<i>ts</i> 662 (I)	7					1 × 10 ⁶	7.0 × 10 ²
	Ad2 + <i>ts</i> 662	9 + 7		2.8 × 10 ⁶	1.0 × 10 ⁶			
	<i>ts</i> 663 (II)	7					5.0 × 10 ⁵	1.6 × 10 ²
	Ad2 + <i>ts</i> 663	9 + 7		2.0 × 10 ⁶	5.6 × 10 ⁴			
	<i>ts</i> 640 (III)	5				2.2 × 10 ²	1.4 × 10 ⁶	1.7 × 10 ³
	Ad2 + <i>ts</i> 640	9 + 5		5.2 × 10 ⁶	1.5 × 10 ⁴			

For the experiment 1, confluent monolayer cultures of CV-1 line of AGMK containing about 2×10^6 cells per 5-cm glass Petri dish were singly or doubly inoculated with 0.5 ml of Ad2, parental SV40 (strain SV40-1), or SV40 *ts* mutants, *ts* 662 (complementation group I) or *ts* 663 (group II). After adsorption at 33°C for 1 h, cultures were washed three times with medium, covered with 4 ml of Dulbecco-modified Eagle's medium containing 5% calf serum and 50 Uml⁻¹ of mycostatin, and incubated at 33°C ± 1°C or 40°C ± 0.5°C in humidified incubators flushed with a CO₂-air mixture. At specified times, cells were collected with medium (two cultures for each point) and stored frozen. After being frozen-thawed more than five times, virus infectivity in the lysates was assayed by plaque formation on cultures of fl-8 line of AGMK transformed by adeno 7-SV40 hybrid virus (E46⁺) (manuscript in preparation) at 37°C (Ad2) and on cultures of BSC-1 line (subline B) of AGMK at 33°C (SV40 and its *ts* mutants). In experiments 2 and 3, confluent 5-cm (experiment 2) or 3-cm (experiment 3) plastic Petri dish cultures containing about 2×10^6 or 7×10^5 cells, respectively, of tertiary AGMK cells or BSC-1 line (subline P66) of AGMK were singly or doubly inoculated with virus. The parental SV40 used was the strain SV68C. After adsorption at 33°C for 1.5 h cultures were treated with anti-SV40 rabbit immune serum for 30 min to remove unadsorbed SV40 and *ts* mutants of SV40 followed by two washings with medium. Cultures were covered with 5 ml (experiment 2) or 2 ml (experiment 3) of Dulbecco-modified Eagle's medium containing 10% foetal bovine serum and 50 Uml⁻¹ of mycostatin. Incubation and collection were the same as in the experiment 1. Virus-infectivity was determined by plaque formation on tertiary cultures of human embryonic kidney cells at 37°C in the presence of anti-SV40 antiserum (Ad2) and on cultures of BSC-1 line (subline B) of AGMK at 33°C (SV40 and *ts* mutants of SV40).

of BSC-1 line (subline P66) of AGMK. This strongly indicates that the SV40 gene controls, either directly or indirectly, the replication of human adenovirus in simian cells. Jerkofsky and Rapp¹⁴ reported that another SV40 early mutant (*ts*A7) can enhance the adenovirus replication at the restrictive temperature. Whether these two early mutants, *ts*A7 and my *ts*640, represent different genes remains to be seen. Since there has been a suggestion that the helper function is correlated with the ability of the cells to be induced to synthesise cellular DNA by infection with SV40¹⁴, it will be interesting, in a future study, to determine whether SV40 and *ts*640 induce cellular DNA synthesis in BSC-1 cells as well as in tertiary AGMK cells. In experiment 2 of Table 2, and occasionally in other experiments, the inhibition of replication of *ts*640 in a single infection of BSC-1 (P66) cells at 40°C was incomplete, in contrast to the complete inhibition in parallel experiments in tertiary AGMK cells (Table 2, experiment 2) or in CV-1 cells (my unpublished observations), suggesting the possibility, among other things, that the cistron III product of SV40 is modified by some cellular factor to carry out its function.

The question is raised: what is the nature of the SV40 gene function identified in mutant *ts*640? A previous study¹² showed that the affected gene was responsible for the synthesis of infectious viral DNA in cells of the BSC-1 line (subline P66) of AGMK and also for the initiation but not for the maintenance of morphological transformation of rat 3Y1 cells. It can be argued that *ts*640 is a double (or triple) mutant but otherwise the gene product affected has three apparently different functions, one in rat cells for the initia-

tion of transformation and the other two in simian cells for the replication of viral DNA and for the facilitation of cell infection by adenovirus. The precise roles of the gene function in each situation and accordingly the relationship between the situations are uncertain. The SV40 mutant defective in these functions described here should prove useful both in understanding adenovirus defectiveness and in defining specific mechanism controlling SV40 DNA replication and cell transformation.

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No crossing over between closely linked tumour virus loci in the heterogametic sex of fowl

RECESSIVE autosomal genes, a^+ , b^+ and c^+ , control the resistance of fowl to Rous sarcoma virus (RSV) of subgroups A, B and C respectively¹⁻⁶. Evidence of a complete or an incomplete linkage between the tumour virus a (tva) and tumour virus c (tvc) loci has been reported⁷ and studies on an F₂ population derived from a cross between C/O (chickens susceptible to subgroup A and subgroup C tumour viruses), and C/AC

The F₁ parents of $a^+a^+c^+c^+$ genotype were derived from a cross between the C/O and C/AC parents. Two test cross (TC) matings were set up. In one, a double dominant heterozygote $a^+a^+c^+c^+$ F₁ male was back-crossed with eight double recessive homozygote, $a^-a^-c^-c^-$ females (TC₁) and in the other eight $a^+a^+c^+c^+$ F₁ females were backcrossed with one $a^-a^-c^-c^-$ male (TC₂). A detailed report on these genotypes and the development of the F₁ and C/AC populations is published elsewhere⁸.

Secondary monolayer cultures of chick embryo fibroblast (CEF) from 11-d-old embryos were individually challenged in pairs with BS-RSV of subgroup A, RSV (RAV49) of subgroup C, and an equal mixture of the two viruses in the same inoculum. Plates were challenged with 0.2 ml of virus suspension diluted to contain 1000 f.f.u. ml⁻¹. Ten days after challenge foci were counted to ascertain resistant and susceptible phenotypes. The detailed procedure of recognition of the four possible subclass phenotypes within dam families and on a population basis, namely C/A (chickens resistant to subgroup A but susceptible to subgroup C); C/C, (chickens resistant to subgroup C but susceptible to subgroup A); C/O, (chickens susceptible to both subgroups) and C/AC, (chickens resistant to both subgroups), is published elsewhere⁸. In designating the phenotypes, the cultures with a focus count of 15 or less were allocated to the resistant class and those above 15 were assigned to the susceptible class.

The distribution of the four subclass phenotypes within dam families and on a population basis for the TC₁, TC₂ and F₂ is shown in Table 1. Based on the observed and expected four subclass phenotype frequency ratios, that is,

TABLE 1 Distribution of four subclass phenotypes within dam families and on a population basis for TC₁, TC₂ and F₂ cultured cells inoculated with BS-RSV, RSV(RAV49) and an equal mixture of the two viruses

Pheno- type	TC ₁ (F1 ♂ × C/AC ♀ ♀) (<i>a⁺a⁺c⁺c⁺</i> × <i>a⁻a⁻c⁻c⁻</i>)									Mating TC ₂ (F1 ♀ ♀ × C/AC ♂) (<i>a⁺a⁺c⁺c⁺</i> × <i>a⁻a⁻c⁻c⁻</i>)									F ₂ (F1 ♂ × F1 ♀ ♀) (<i>a⁺a⁺c⁺c⁺</i> × <i>a⁻a⁻c⁻c⁻</i>)									Total pheno- type
	Dam No.								Total pheno- type	Dam No.								Total pheno- type	Dam No.								Total pheno- type	
	20	21	22	23	24	25	26	27		270	271	272	273	274	275	276	277		270	271	272	273	274	275	276	277		
C/O	3	4	4	1	3	4	3	2	24	5	6	1	2	3	3	6	—	26	8	9	4	6	4	6	8	7	52	
C/A	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	—	0	1	0	1	0	0	0	0	0	2	
C/C	0	2	2	0	1	2	1	0	8	0	0	0	0	0	0	0	—	0	0	0	2	0	2	0	0	0	4	
C/AC	3	5	0	3	3	2	2	1	19	2	3	0	0	1	4	2	—	14	1	1	3	3	1	3	2	3	17	
Total	7	11	6	4	7	8	6	3	52	7	9	1	2	4	7	8	—	40	10	10	10	9	7	9	10	10	75	

(chickens resistant to both subgroups) parents favoured incomplete linkage of these two loci⁸. The linkage value computed from the results of the F₂ population was 0.08 ± 0.03 based on an assumption of equal crossing over in gametes produced by the male and female parents. This report describes the distribution of segregants arising from two reciprocal double back crosses (F₁ ♂ × C/AC ♀ ♀ and F₁ ♀ ♀ × C/AC ♂), which strongly suggests the absence of crossing over in the heterogametic sex (female).

1 C/O: 1 C/A: 1 C/C: 1 C/AC: the χ^2 analysis⁹ for the TC₁ is shown in Table 2. A non-random segregation and re-association of the genes at the tva and tvc loci was indicated by the highly significant χ^2 value, 25.08 ($P < 0.01$). The single degree χ^2 analysis⁹ suggested linkage between the tva and tvc loci, because the segregations of a^+a^+ and c^+c^+ genes were not significant ($P > 0.05$) in contrast to a highly significant χ^2 value, 22.23 ($P < 0.01$), for the joint segregation component (Table 2).

TABLE 2 χ^2 linkage test for detection of linkage between tva and tvc loci in the TC₁ population and segregation analysis of the phenotypes based on $\dagger p = 0.5$ and 0.17

Genotype	Phenotype	Observed number	Expected frequency	Expected No.				χ^2 deviation				χ^2 linkage test	
				$p = 0.5$	$p = 0.17$	$p = 0.5$	$p = 0.17$	$p = 0.5$	$p = 0.17$	$p = 0.5$	$p = 0.17$	$p = 0.5$	$p = 0.17$
$a^+a^+c^+c^+$	C/O	24	$\frac{1}{2}(1-p)$	13.00	21.32	9.31	0.02	segregation, a^+a^+ segregation, c^+c^+ joint segregation (linkage)		d.f. 1 1 1		χ^2 2.77* 0.08* 22.23†	
$a^+a^+c^-c^-$	C/A	1	$\frac{1}{2}(p)$	13.00	4.68	11.08	2.89						
$a^+a^+c^+c^+$	C/C	8	$\frac{1}{2}(p)$	13.00	4.68	1.92	2.36						
$a^-a^-c^+c^+$	C/AC	19	$\frac{1}{2}(1-p)$	13.00	21.32	2.77	0.25						
Total		52	1.00	52.00	52.00	25.08†	5.52*						

* Significance level; $P > 0.05$.

† Significance level; $P < 0.01$.

‡ $p = 0.5$ that is, random reassociation of non-allelic pairs of genes at the tva and tvc loci.

TABLE 3 χ^2 linkage test for the detection of linkage between the *tva* and *tvc* loci in the TC₂ population and segregation analysis of the phenotypes based on $p = 0.5, 0.17$ and 0.00

Genotype	Phenotype	Observed No.	Expected frequency	Expected No.			χ^2 deviation			χ^2 linkage test $p = 0.5$	d.f.	χ^2
				$p = 0.5$	$p = 0.17$	$p = 0.00$	$p = 0.5$	$p = 0.17$	$p = 0.00$			
$a^+a^+c^+c^+$	C/O	26	$\frac{1}{2}(1-p)$	10	16.60	20.00	25.60	5.32	1.80	Segregation $a^+:a^-$	1	3.60*
$a^+a^+c^-c^-$	C/A	0	$\frac{1}{2}(p)$	10	3.40	0	10.00	3.40	0	Segregation $c^+:c^-$	1	3.60*
$a^+a^+c^+c^-$	C/C	0	$\frac{1}{2}(p)$	10	3.40	0	10.00	3.40	0	Joint segregation (linkage)	1	40.00†
$a^+a^+c^-c^+$	C/AC	14	$\frac{1}{2}(1-p)$	10	16.60	20.00	1.60	0.41	1.80			
Total		40	1.00	40	40.00	40.00	47.20†	12.53†	3.60*		3	47.20†

* Significance level; $P > 0.05$.† Significance level; $P < 0.01$.

Nine out of fifty-two were cross over phenotypes (C/A + C/C), and by the maximum likelihood method⁹ the linkage value (p) was estimated to be 0.17 ± 0.05 . On the basis of this p value, the observed phenotype frequencies did not deviate significantly from those of the expected ones ($\chi^2 = 5.52$, $P > 0.05$, Table 2).

The χ^2 analysis for the TC₂, based on observed and expected four subclass phenotypes in a ratio of 1:1:1:1, also gave strong support for linkage between the *tva* and *tvc* loci (Table 3). No cross-over phenotype of either C/A or C/C, out of 40 embryos tested was observed however. It is also possible that very little crossing over had occurred in the TC₂, and that by chance the cross-over products did not appear in this population. This supposition is not testable as p is unknown. Under the assumption of equal p in either TC, that is, $p = 0.17 = TC_1 = TC_2$, the χ^2 value, 12.53 ($P < 0.01$) significantly rejected this postulate (Table 3). Alternatively on the basis of $p = 0.00$, the χ^2 analysis presented in Table 3 favoured the observed 1:1 segregation ($\chi^2 = 3.60$, and $P > 0.05$), which suggests the absence of crossing over in the TC₂.

In the absence of linkage between the *tva* and *tvc* loci, four types of gametes, a^+c^+ , a^+c^- , a^-c^+ and a^-c^- should have been produced by male and female parents at equal frequencies during gametogenesis in the TC₁ and TC₂ respectively. But because of linkage between the two loci the expected frequency distribution of the gametic array was disturbed in proportion to the crossing over between the non-allelic pairs of genes. As a result of differential crossing over in the gametes produced by double heterozygote males of the TC₁ and females of the TC₂, 17% of crossing over was observed in the TC₁ population compared to none in the TC₂. These results and their appropriate χ^2 analysis strongly imply the absence of crossing-over in the heterogametic sex.

This concept was further analysed on the results of the distribution of subclass phenotypes of the F2 population reported earlier⁸. The observed and expected segregation of the four subclass phenotypes of the F2 population is shown in

Table 4. With $p = 0.5$, the χ^2 value was highly significant ($P < 0.01$), indicating a non-random reassociation of genes at the *tva* and *tvc* loci and the linkage test shown in Table 4 confirmed that the *tva* and *tvc* loci are linked ($\chi^2 = 51.81$, $P < 0.01$). In our previous report the results were analysed under the assumption of an equal crossing over in the male and female gametes and the χ^2 analysis supported this concept. Accordingly the p value was estimated to be 0.08 ± 0.03 . The predicted frequency distribution of the four subclass phenotypes, C/O, C/A, C/C and C/AC based on the p value estimated in this study, (0.17 for male and 0.00 for female) is also presented in Table 4. Because the χ^2 value, 0.86 was not significant ($P > 0.05$), the proposed concept of the absence of crossing over in the heterogametic sex of fowl is upheld. The overall p value 0.10 ± 0.03 estimated from the two reciprocal double back crosses, agreed well with that reported previously and by combining the data of the TC₁, TC₂ and F2 populations the best joint estimate of p was 0.09 ± 0.02 . Results obtained from other crosses also support the present findings and will be published elsewhere.

Thus from the results of TC₁, TC₂ and F2 two important conclusions can be drawn: (1) the *tva* and *tvc* loci are linked with a linkage value of 0.17 ± 0.05 , (2) very little or no crossing over occurs between the *tva* and *tvc* loci in the heterogametic sex.

It is an established hypothesis that in many species the heterogametic sex has a strong influence on crossing over during gametogenesis¹⁰. In *Drosophila* the mutant, *c(3)G* in chromosome III has been shown to suppress crossing over in the male¹²; but it is not known whether similar mutants exist in other species.

In fowl, however, sex has little effect on crossing over in all linkage groups except the rose comb-creeper pair¹⁰⁻¹¹. In the latter, crossing over was observed more than twice as frequently in the females as in the males. The results in this study on the other hand, suggest the contrary because very little or no crossing over occurred between the *tva* and *tvc* loci in the female compared with 17% in the male. Because in the fowl the female is the heterogametic sex, the result

TABLE 4 χ^2 linkage test for the detection of linkage between the *tva* and *tvc* loci in the F2 population and segregation analysis of phenotypes based on $p \sigma = 0.17$ and $p \varnothing = 0.00$

Genotype	Phenotype	Observed No.	Expected frequency		Expected No.		χ^2 deviation		χ^2 linkage test $p = 0.5$	d.f.	χ^2
			$p = 0.5$	$p \sigma = 0.17$ $p \varnothing = 0.00$	$p = 0.5$	$p \sigma = 0.17$ $p \varnothing = 0.00$	$p = 0.5$	$p \sigma = 0.17$ $p \varnothing = 0.00$			
a^+c^+	C/O	52	0.5625	0.7050	42.187	52.875	2.28	0.01	Segregation, $a^+:a^-$	1	0.00*
a^+c^-	C/A	2	0.1875	0.0450	14.063	3.375	10.35	0.56	Segregation, $c^+:c^-$	1	0.36*
a^-c^+	C/C	4	0.1875	0.0450	14.063	3.375	7.20	0.12	Joint segregation (linkage)	1	51.81†
a^-c^-	C/AC	17	0.0625	0.2050	4.687	15.375	32.34	0.17			
Total		75	1.0000	1.0000	75.000	75.000	52.17†	0.86*		3	52.17†

* Significance level; $P > 0.05$.† Significance level; $P < 0.01$.

in this study therefore supports the present hypothesis that heterogametic sex influences crossing over. In view of the inconsistent sex effect in other linkage groups an explanation of the sex influence on crossing over in the *tva-tvc* linkage pair is difficult without further studies. It may be that the F1 females of the strain of fowl under study are carrying structural chromosomal changes, such as inversion and translocation, which could influence crossing over indirectly¹². A careful cytological study might show such changes.

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Scrapie in sheep selectively bred for high susceptibility

THE enigma of scrapie disease of sheep is still unresolved, in spite of intensive efforts—especially over the past 12 yr—to elucidate its pathogenesis and to define the transmissible agent. Various hypotheses on the nature of scrapie remain unproved¹⁻¹⁰.

It has long been recognised that heredity is an important factor in the transmission of naturally-occurring scrapie. In 1961 a selective breeding programme with Herdwick sheep was started at this Institute, with the objective of developing from the same foundation stock, two flocks, one highly

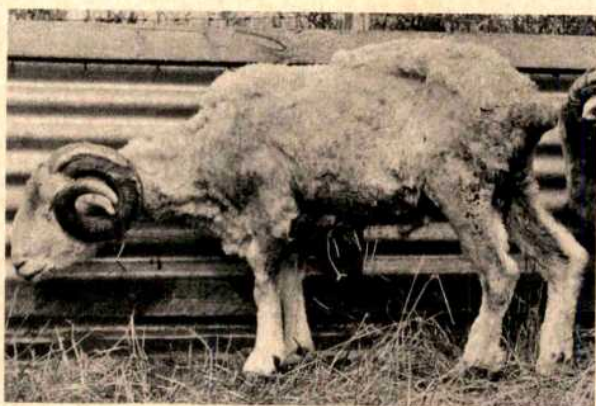


Fig. 1 Case A: a scrapie-affected ram. Note dullness, rubbed areas and hind-limb weakness.

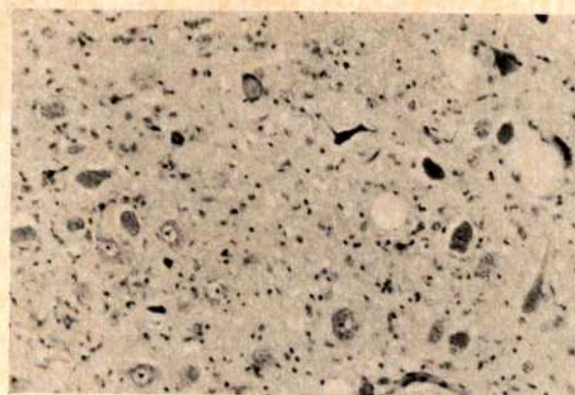


Fig. 2 Case A: a histological section of the medulla oblongata. There is extensive spongiform degeneration of the 10th nucleus. Stained with haematoxylin and eosin (X25).

susceptible and the other highly resistant to experimental scrapie. This objective has now been achieved¹¹. Briefly, in two successive years 40 rams and 699 ewes were mated in forty separate flocks to produce two crops of lambs. One crop of lambs and their parents were then challenged with scrapie by inoculation. A proportion of the inoculated animals developed scrapie during the following 2 yr. The non-inoculated crop of offspring could then be defined in terms of the susceptibility to scrapie of their parents and their sibs. Animals of which the parents and sibs developed scrapie were the foundation stock for the present flock that is highly susceptible to experimental scrapie; likewise, the foundation stock of the highly resistant flock had parents and sibs that did not develop scrapie.

The breeding part of this programme has been carried out exclusively in five buildings well separated from each other on a restricted and clearly defined experimental area of the 2,000 acre estate that is part of this Institute. Neither natural nor experimental scrapie had ever been present in this area before the occurrence of the two cases of the disease described here. Sheep to be inoculated with scrapie have always been removed to one of two other clearly defined areas on the estate, each approximately 1 mile away. Different shepherds have looked after the breeding flocks and infected flock, respectively. The isolation of the breeding flocks has been strictly maintained.

Both the cases of scrapie that are the subject of this communication have occurred in the flock that has been bred for high susceptibility to experimental scrapie. The first (case A) was in July 1972 in a ram 40 months old. Clinical signs of scrapie were typical, and included rubbing over the rump, hind-limb incoordination and weakness, and a dull facial expression (Fig. 1). The second (case B) was in November 1973 in a ewe 43 months old. Again, the clinical signs were typical and closely similar to those of case A. Both animals were observed for a few days and were then killed for *post mortem* examination. In both cases histological examination of the brain showed in the medulla oblongata the characteristic bilaterally symmetrical spongiform encephalopathy of scrapie, with vacuolation of the neurones; spongiform change was particularly marked in the region of the 10th nucleus (Fig. 2). Neurones showing foamy vacuolation (Fig. 3) were present in both cases. This type of vacuolation is typical of natural scrapie, and is rare in the experimentally produced disease¹². Also, in both cases vacuolated neurones were more obvious and more numerous than is usual in the experimental disease^{12,13}.

Because the histopathology of these two cases was that of naturally-occurring scrapie, a histological re-examination was made of the 99 cases of scrapie that have occurred in

Herdwick sheep inoculated at this Institute over the past 6 yr with a standard dose of a pool of homogenised scrapie sheep brain. A single section of the medulla oblongata close to the calamus scriptorius¹⁴ from each of these 99 brains was compared with single sections of the same area from cases A and B. Particular attention was paid to vacuolated neurones, foamy vacuolation of neurones and spongiform vacuolation of the 10th nucleus. The results are shown in Table 1. These results indicated strongly that the scrapie of cases A and B differed from the experimental scrapie produced by the standard inoculum. Because scrapie can still be defined only in clinicopathological terms, no other test was available to differentiate between these two expressions of the disease. It may be noted, however, that both cases occurred at ages (40 and 43 months) close to the mean of 39 months in the highly characteristic age incidence pattern recorded by Parry² for 1,008 cases of naturally-occurring scrapie.

Scrapie cases A and B both occurred in the flock bred for high susceptibility to experimental scrapie. Although they had four common ancestors, they were not very closely related to each other. The coefficient of inbreeding¹⁵ of case A was 0.063, and of case B was 0.008.

The mechanism of the spread of scrapie disease has been extensively studied.¹⁶ It is generally accepted that natural scrapie is usually perpetuated in a hereditary manner, and the very rare exceptions in which there has apparently been lateral spread may have been related to ingestion of foodstuff contaminated by foetal membranes voided by a scrapie-affected ewe at lambing time¹⁷. The experimental disease does not spread by contact, unless—as may happen with rodents—there has been ingestion of scrapie tissue. It is extremely unlikely, therefore, that scrapie cases A and B could have occurred by either direct or indirect contact with natural or experimental scrapie. They were separated in time by 16 months, and the physical organisation of the experimental programme makes it impossible that they were accidentally inoculated with scrapie; even had this been so, the histopathology would have been that of the experimental disease.

I conclude that these two cases of scrapie arose spontaneously by genetic selection in a breeding programme designed to increase susceptibility to experimental scrapie. This conclusion supports the proposition put forward by Parry² that in the naturally-occurring disease the scrapie agent can be formed within susceptible sheep, and it agrees with the long-established fact that scrapie is frequently associated with attempts to improve the conformation and performance of sheep by inbreeding¹⁸.

A genetic basis for the development of scrapie in sheep is consistent with observed facts, but it leaves unresolved the problem of the nature of the transmissible agent. A majority of investigators have assumed that the agent is self-replicating because its titre in body tissues rises during sub-clinical and clinical development of the disease. Self-replication implies involvement of nucleic acid. Nucleic acid, however, has not been identified, and there is impressive experimental evidence from ultraviolet irradiation studies



FIG. 3 Case B: a histological section of the medulla oblongata showing foamy vacuolation of a neurone. Stained with haematoxylin and eosin (X105).

that it may not be present in the agent¹⁹⁻²². Pattison and Jones⁵ pointed out that if the scrapie agent were present in normal tissues in an inhibited form and if it were gradually released as scrapie developed, this unmasking would provide an alternative explanation to self-replication. It seems possible that a mechanism such as this could be genetically controlled, and that resultant disorganisation of metabolism could cause degeneration of the central nervous system.

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TABLE 1 Comparison of single sections of the medulla oblongata from 99 cases of experimental scrapie with those from cases A and B.

Group	Number of cases showing:		
	Vacuolated neurones	Foamy vacuolation of neurones	Spongiform vacuolation of the 10th nucleus
99 cases of experimental scrapie	30	0	0
Scrapie Cases A and B	2	2	2

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Anti-muscarinic properties of neuroleptics and drug-induced Parkinsonism

ONE unifying hypothesis of the behavioural effects produced by neuroleptic drugs is that they act by blocking dopamine receptors in the central nervous system¹⁻³. Neuroleptic drugs produce increases in dopamine turnover⁴, increase accumulation of its metabolites⁵, antagonise amphetamine induced behaviour in man and animals⁶ and block the stimulating effects of dopamine on cyclic AMP production in homogenates of rat brain striatum or mesolimbic nuclei⁷⁻⁹.

One complication in the use of these drugs is that they often produce severe extrapyramidal side effects including a syndrome similar to idiopathic Parkinsonism^{10,11}. This syndrome has been explained as a blockade of dopamine receptors by the drugs resulting in a functional lack of dopamine in the basal ganglia. The extrapyramidal side effects may be treated by centrally acting anticholinergic agents¹² which restore the balance between the antagonistic dopaminergic and cholinergic influences in the basal ganglia¹¹ without interfering with the antipsychotic actions of the neuroleptics.

It has been shown⁸ that a series of neuroleptics differed over a 50-fold range in their ability to inhibit the striatal dopamine-sensitive adenylyl cyclase (Table 1). The more potent antidopaminergic drugs, however, are used clinically in much lower doses than the less potent drugs and on this basis one might therefore expect all neuroleptics to produce about the same incidence of extrapyramidal signs. That this is not the case has become increasingly apparent. In particular the phenothiazine, thioridazine, and the recently described dibenzodiazepine, clozapine, have been shown to produce very few extrapyramidal motor side effects¹³⁻¹⁵. Clozapine has been shown to be an anticholinergic agent using the guinea pig ileum and it has been suggested that the paucity of extrapyramidal side effects seen with this drug was a result of this property¹⁶⁻¹⁸. In the present experiments we have used a specific radioactive affinity label for muscarinic receptors to determine if any relationship exists between drug induced Parkinsonian signs and anti-muscarinic activity in neuroleptic drugs.

Male Wistar rats were decapitated and their brains removed. The cerebral cortex was homogenised in 10 volumes of ice cold 0.32 M sucrose. Protein concentration was determined by the method of Lowry *et al.*¹⁹ and the homogenate was diluted in Krebs-Henseleit solution (equilibrated with 5% CO₂ in oxygen) to give 0.27 mg protein ml⁻¹. 3 ml aliquots of homogenate were preincubated for 15 min with the drug being tested and then the aziridinium ion from N-2'-chloroethyl-N-[2'', 3'', ³H]-propyl-2-aminoethylbenzi-

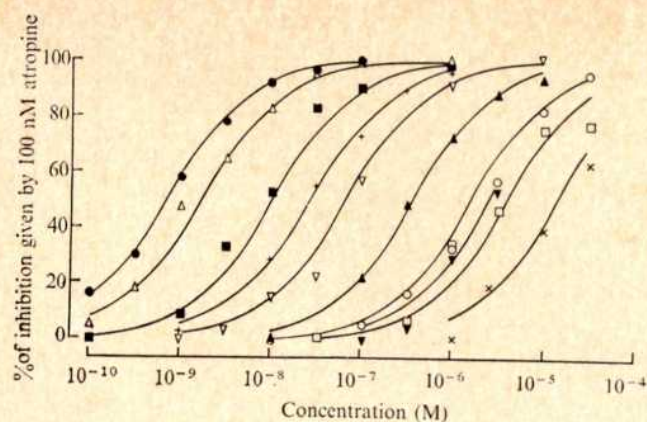


FIG. 1 Inhibition of atropine-sensitive uptake of ³H-PrBCM by neuroleptic agents and other drugs. Experiments were performed in quadruplicate and standard errors were < 10%. The lines are theoretical mass action curves derived using the dissociation equilibrium constants given in Table 1. Atropine-sensitive uptake was approximately 60% of a total uptake of 700 pmol ³H-PrBCM per g protein. ●, Atropine; Δ, benztropine; ■, ethopropazine; +, thioridazine; ▽, clozapine; ▲, chlorpromazine; ○, pimo- zide; ▼, flupenthixol; □, trifluoperazine; ×, spiroperidol.

late (³H-PrBCM: 1.8 Ci mmol⁻¹) added to give a final concentration of 2 × 10⁻⁹ M. The aziridinium ion was prepared as described by Burgen *et al.*²⁰. Incubation was continued for 8 min and was stopped by the addition of 20 ml Krebs-Henseleit solution containing 10 mM sodium thio- sulphate. Samples were filtered under suction through Whatman GF/C glass fibre filters, washed with 20 ml of Krebs-Henseleit solution and the tritium remaining on the filters determined by liquid scintillation counting.

The concentration of ³H-PrBCM used produced 88% receptor occupation in an uninhibited reaction. Percentage inhibition of the rate constant of alkylation was determined by the method of Burgen *et al.*²¹. Dissociation equilibrium constants were derived from the slope of the plot (fractional inhibition of rate constant)⁻¹ against (concentration of reversible inhibitor)⁻¹.

The effect of different drug concentrations on the atropine-sensitive binding of ³H-PrBCM in cortical homogenates is shown in Fig. 1. Dissociation equilibrium constants for the various drugs are listed in Table 1. The most potent drug used was the classical antimuscarinic agent, atropine, which was included for comparison. It is clear that the neuroleptics differ over a wide range (approximately four orders of magnitude) in their antimuscarinic potencies. The results obtained here support the hypothesis that the antimuscarinic activity of these drugs is related to the frequency of extra- pyramidal side effects. For example, in considering drugs of the phenothiazine class one finds a spectrum of effects. These drugs range from trifluoperazine, a potent dopamine receptor blocker with little antimuscarinic activity, that causes a high frequency of extrapyramidal effects, through chlorpromazine and thioridazine to ethopropazine, a drug with no neuroleptic effects, which has in fact been used as an anti-Parkinsonian agent¹¹. The antimuscarinic effect of benztropine, a classical anticholinergic, anti-Parkinsonian agent¹¹ is shown for comparison. The incidence of extra- pyramidal effects found with the other drugs is also in- versely related to their antimuscarinic activity. Spiroperidol, a butyrophenone and flupenthixol, a thioxanthene, have weak antimuscarinic effects and produce frequent side ef- fects¹³. Clozapine produces few side effects and is a con- siderably more potent antimuscarinic agent.

Several other observations concerning the behavioural and metabolic effects of neuroleptics may also be interpreted in the light of these results. Thus, clozapine causes a smaller

TABLE 1 Dissociation equilibrium constants of various drugs for cortical muscarinic receptors

Drug	Dissociation equilibrium constant (M)	IC ₅₀ for striatal* dopamine-sensi- tive adenylyl cyclase (M)
Atropine	5.2 ± 0.3 × 10 ⁻¹⁰	—
Benztropine	1.3 ± 0.1 × 10 ⁻⁹	> 10 ⁻⁴
Ethopropazine	1.0 ± 0.1 × 10 ⁻⁸	—
Thioridazine	2.5 ± 0.1 × 10 ⁻⁸	3.0 × 10 ⁻⁶
Clozapine	5.5 ± 1.0 × 10 ⁻⁸	4.5 × 10 ⁻⁶
Chlorpromazine	3.5 ± 0.2 × 10 ⁻⁷	1.0 × 10 ⁻⁶
Pimozide	1.6 ± 0.1 × 10 ⁻⁷	4.5 × 10 ⁻⁶
Trifluoperazine	4.0 ± 0.4 × 10 ⁻⁶	4.0 × 10 ⁻⁷
Flupenthixol	2.2 ± 0.8 × 10 ⁻⁶	7.0 × 10 ⁻⁸
Spiroperidol	1.2 ± 0.1 × 10 ⁻⁵	2.0 × 10 ⁻⁶

The dissociation equilibrium constants are determined as in the text. The effect of some of these compounds on striatal dopamine-sensitive adenylyl cyclase is shown for comparison.

* Data from Miller and Iversen, 1974⁸.

increase in homovanillic acid accumulation in basal ganglia than in the limbic system of rabbits¹⁷ and thioridazine produces little increase in homovanillic acid accumulation in monkey basal ganglia¹. In addition, it has recently been suggested that the different effects of thioridazine and chlorpromazine in antagonising amphetamine-induced turning in rats, argue against dopamine receptor blockade being involved in neuroleptic activity²². These results, however, may be explained by the more potent antimuscarinic activity of thioridazine.

In conclusion, the results presented here support the hypothesis that neuroleptic drugs with potent antimuscarinic activity produce few Parkinsonian side effects. Combination of the method used here with an *in vitro* measure of dopamine receptor blockade, such as the dopamine-sensitive adenylyl cyclase, should ensure improved and rapid selection of potentially useful neuroleptic agents.

In the course of this study we learned of a similar series of experiments performed by Snyder, Greenberg and Yamamura²³. The results of their work seem to agree with those reported here.

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Inhibition of normal clotting and Fletcher factor activity by rabbit anti-kallikrein antiserum

NORMAL human plasma clots rapidly on contact with a foreign surface such as glass, through a sequence of reactions designated the intrinsic pathway of thrombin formation. Two nearly identical mechanisms, designated a waterfall¹ and cascade² respectively, were proposed to explain this process. Although these hypotheses have since been modified³, ideas about the clotting factors involved and the essential order of their participation have not changed. Many of the reactions have been clarified through the use of plasma from patients with congenital deficiencies and partially purified preparations of clotting factors.

Hathaway *et al.*⁴ described a family in whom impaired blood clotting seemed to be due to the deficiency of a hitherto unrecognised clotting factor. The defect, designated Fletcher factor deficiency or Fletcher trait, after the affected kindred, has been recognised in at least three further patients⁵. Wuepper⁶ reported that Fletcher trait plasma is functionally and antigenically deficient in a plasma prekallikrein, and that the addition of purified preparations of prekallikrein corrected the clotting defect of Fletcher trait plasma. This observation, confirmed in this and other laboratories^{7,8}, suggests that a plasma prekallikrein or its activated form, kallikrein, participates in normal blood clotting. To examine this possibility further, we have prepared antiserum in rabbits against purified human plasma kallikrein and examined its effect on the clotting of normal human plasma. We report here that this anti-kallikrein antibody impedes the intrinsic pathway of thrombin formation by depleting normal plasma of Fletcher factor activity.

Partially purified human plasma kallikrein was prepared from human oxalated plasma by tricalcium phosphate adsorption, Celite 512 adsorption and elution, and successive column chromatography on SP-Sephadex C-50, QAE-Sephadex A-50 and Sephadex G-150 (Pharmacia, New Market, New Jersey)⁹. The preparation released 26.4 μ M methyl alcohol (MeOH) $\text{ml}^{-1} \text{h}^{-1}$ (specific activity: 775 μ M MeOH mg^{-1} protein h^{-1}) from *p*-toluenesulphonyl-L-arginine methyl ester¹⁰ and approximately 250 ng of bradykinin per ml in the rat uterus assay¹¹. The kinin generating activity was inhibited by soybean trypsin inhibitor (0.5 mg ml^{-1}) and Trasylol (500 U ml^{-1}), but not by lima bean trypsin inhibitor (0.5 mg ml^{-1}). It contained traces of activated PTA activity (less than 0.005 U ml^{-1}), but no measurable amount of other known clotting factors or plasmin.

TABLE 1 Effect of anti-kallikrein antibody on screening tests of blood coagulation

Test	Clotting time	
	Normal rabbit γ G + normal plasma	Anti-kallikrein antibody + normal plasma
	(s)	(s)
Thrombin time*	31.4	32.8
One-stage prothrombin time	16.8	16.8
Russell's viper venom time	11.9	12.6
Kaolin-activated partial thromboplastin time†	67.0	298.4

A sample of 0.4 ml normal human pooled plasma was incubated with equal amounts of either normal rabbit γ G (21 mg protein per ml) or anti-kallikrein antibody (33 mg protein per ml) in a 10 \times 75 mm polystyrene tube at 37° C for 60 min. The mixture was then tested. The techniques used in these tests have been summarised before¹².

* 2.5 NIH U/ml crude bovine thrombin (Parke, Davis, Detroit).

† 0.1 ml test sample was incubated with 0.1 ml kaolin-Gliddex-P in a 10 \times 75 mm glass tube at 37° C 1 min before adding 0.1 ml 0.025 M CaCl₂.

Rabbit antiserum to human plasma kallikrein was obtained by injecting a mixture of equal parts of partially purified kallikrein, containing approximately 50 μ g protein, and alumina C γ -gel (A grade, Calbiochem), into the footpads of New Zealand albino female rabbits. Four weeks later

TABLE 2 Effect of anti-kallikrein antibody on some clotting factors in the intrinsic pathway

Clotting factor	Dilution of anti-kallikrein or normal rabbit γ G	Clotting time			
		Normal rabbit γ G + normal plasma		Anti-kallikrein antibody + normal plasma	
		(s)	(%)	(s)	(%)
Hageman factor	Undiluted	66.2	100	68.6	86
PTA	Undiluted	121.5	100	116.6	105
Christmas factor	Undiluted	120.9	100	121.5	100
AHF	Undiluted	111.5	100	112.8	100
Fletcher factor	Undiluted	103.6	100	378.2	<1
Fletcher factor	1/2	—	—	378.8	<1
Fletcher factor	1/4	—	—	335.7	<1
Fletcher factor	1/8	—	—	224.6	2.5

A sample of 0.05 ml normal human pooled plasma was incubated with equal amounts of either normal rabbit γ G (21 mg protein per ml) or various dilutions of anti-kallikrein antibody (33 mg protein per ml) in 10 \times 75 mm polystyrene tubes at 37° C for 60 min. Then, 1.9 ml ice-cold barbitol-saline buffer (0.025 M barbitol-sodium barbitol, 0.125 M sodium chloride, pH 7.5) was added to each tube and 0.1 ml aliquots were assayed for each clotting factor listed above. The assays of Hageman factor, PTA, Christmas factor and AHF were performed as described before¹⁶ on substrates of plasma from patients with congenital clotting deficiencies. Fletcher factor was assayed by incubating a 0.1 ml test sample with 0.1 ml kaolin-Gliddex-P and Fletcher trait plasma in 10 \times 75 mm glass tubes at 37° C for 1 min. 0.1 ml 0.025 M CaCl₂ was then added to the tubes and the clotting time was measured at 37° C. The results were expressed as the clotting time and as the percentage of activity relative to that in the mixture with normal rabbit γ G.

the rabbits received a further injection of this kallikrein without adjuvant. Antiserum was collected 1 week thereafter. When subjected to immunodiffusion in 0.9% Agarose, the unabsorbed antiserum gave three precipitin lines with normal, Hageman trait or plasma thromboplastin antecedent (PTA)-deficient plasma, but only two lines with Fletcher trait plasma. After absorption with Fletcher trait plasma, the antiserum was adsorbed with tricalcium phosphate, subjected to 60° C for 60 min to inactivate clotting factors, and the γ -globulin fraction was separated by treatment with *n*-octanoic acid and ammonium sulphate¹². As a control, normal rabbit γ -globulin was prepared in the same way. The γ -globulin prepared from absorbed anti-kallikrein serum (anti-kallikrein antibody) formed a single precipitin line of identity against normal, Hageman trait or PTA-deficient plasma, and purified kallikrein or prekallikrein, whereas it formed no recognisable line against Fletcher trait plasma. The absorbed anti-kallikrein antibody retained the ability of unabsorbed antiserum to neutralise the kinin-generating activity of purified plasma kallikrein.

Normal human pooled citrated plasma, prepared from twenty-five normal adult males, was incubated with equal amounts of either normal rabbit γ -globulin or anti-kallikrein antibody at 37° C for 60 min. The mixtures were then tested in screening tests of blood coagulation. The thrombin time, prothrombin time and Russell's viper venom time were the same in both mixtures (Table 1). In contrast, the kaolin-activated partial thromboplastin time (kaolin PTT) was prolonged considerably in a mixture containing anti-kallikrein antibody (298.4 s) compared with that of a mixture containing normal rabbit γ -globulin (67.0 s).

These experiments suggested that the anti-kallikrein anti-

body interfered with an early phase of the intrinsic pathway. Four clotting factors have been recognised as participants of this stage: Hageman factor (factor XII), PTA (factor XI), Christmas factor (factor IX) and antihemophilic factor (AHF, factor VIII). The functional activity of each of these clotting factors, assayed after incubation of normal plasma with anti-kallikrein antibody at 37° C for 60 min, was unaltered, as though the prolonged kaolin PTT was not due to the neutralisation of these clotting factors (Table 2). The Fletcher factor activity of normal plasma, assayed on the substrate of Fletcher trait plasma, however, was reduced sharply by incubation with as little as a four-fold dilution of anti-kallikrein antibody (less than 1% activity compared with a control). These experiments demonstrated that the incubation of normal plasma with anti-kallikrein antibody resulted in the impaired clotting through the intrinsic pathway by depleting its Fletcher factor activity.

The use of anti-kallikrein antibody in this study further supports the view that a plasma kallikrein may be required for the *in vitro* intrinsic pathway of blood coagulation. The exact site at which kallikrein participates in the intrinsic pathway is not yet settled, but evidence has been obtained that kallikrein may be required for the action of the activated Hageman factor¹³. Recently, Gjønnæss presented data suggesting that under certain conditions the extrinsic pathway of blood clotting may also be activated through an action of a plasma kallikrein on factor VII¹⁴. These studies serve once again to emphasize the intimate relationships between different systems of host-defense mechanisms, here the clotting and the kallikrein-kinin system.

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Lectin purification using formalinised erythrocytes as a general affinity adsorbant

MANY carbohydrate-binding proteins, called lectins, purified from plant extracts¹, are proving useful for the study of protein-carbohydrate interactions, cell-surface-initiated differentiation of cells and the topography of cell surface constituents^{1,2}. A general method for purification of lectins would markedly augment this work. Affinity chromatography has been successful for purification of some lectins³ although others require conventional chemical fractionation. Purification may be simplified by the availability of commercial carbohydrate polymer adsorbants: for example Sephadex G-50³ for concanavalin A and Sepharose 4B for the carbohydrate-binding protein from *Dictyostelium discoideum*⁴.

Affinity chromatography can be difficult, however, if adsorbants must be manufactured. Purification of lectins would be easier if a general class of natural insoluble affinity adsorbants were available. Since lectins are generally identified by the ability to agglutinate erythrocytes of an appropriate type, we have tried formalinised erythrocytes as the affinity adsorbant. They have the advantage of being stable, not lysing in hypotonic or hypertonic media or when exposed to high concentrations of lectins. We found that formalinised erythrocytes bind lectins efficiently and release them on addition of appropriate sugars. Our method makes possible preparation of highly purified lectins with a minimum of effort and should prove applicable to all proteins which bind to carbohydrates on cell surfaces.

Crude plant seed extracts (Table 1) were assayed for the ability to agglutinate several types of formalinised erythrocytes⁵, and the type which gave the highest agglutination titre was used for purification. Assays were performed in microtitre V plates (Cooke Engineering) using serial two-fold dilutions of the extracts. Each well contained 25 μ l of lectin solution in 0.15 M NaCl (buffered to pH 6.4 for assays of concanavalin A) and 25 μ l of a 2.5% (v/v) suspension of formalinised erythrocytes in 0.15 M NaCl. Patterns were read 45 min after starting the reaction. The affinity of the erythrocyte adsorbant for lectin was determined by adding various amounts of erythrocytes to crude extract, shaking for 10 min and assaying the adsorbed supernatant for residual lectin activity after centrifugation at 10,000g for 10 min. For all lectins a ratio of erythrocytes to crude extract was chosen such that at least 95% of agglutination activity was adsorbed.

For purification the predetermined amounts of extract and formalinised erythrocytes were combined, and 0.15 M NaCl

was added to bring the final volume to a 30% (v/v) cell suspension, which was incubated at room temperature for 30 min with gentle shaking. The cells were then washed four times with 10 cell volumes of saline with centrifugation at 3,000g for 5 min. The lectin was eluted by shaking the washed cells (30% v/v) in 0.15 M NaCl containing 0.3 M sugar. After 30 min the supernatant was collected by centrifugation at 5,000g for 5 min, recentrifuged at 10,000g for 20 min and then exhaustively dialysed against 0.15 M NaCl to remove the sugar. The formalinised erythrocytes could be washed and used for another purification.

By this procedure, we obtained highly purified lectins in high yield from four plant sources (Table 1). Crude extracts containing concanavalin A and wheat germ agglutinin (WGA), were purified partially before adsorption to erythrocytes, whereas *Ulex* and lima bean lectins were purified directly from crude extracts. The binding capacity of the formalinised erythrocytes varied but was always sufficiently high for efficient purification. Under the conditions used binding was in the range of 0.4–1.7 mg lectin per packed ml of erythrocyte adsorbant and recoveries ranged from 50 to 100%. In all cases substantial quantities of pure lectin could be obtained with small amounts of adsorbant, so that conventional centrifugation facilities are adequate for preparative procedures several orders of magnitude larger than reported here.

The purity of the lectins was determined by polyacrylamide gel electrophoresis in a sodium dodecyl sulphate (SDS) system (Fig. 1). With concanavalin A, WGA and lima bean agglutinin a single protein band was observed after staining. With *Ulex* we found approximately equal amounts of two closely migrating proteins. All molecular weights agreed with previous determinations—WGA, 23,000⁶; lima bean, 30,000⁷; concanavalin A, 25,000⁸. With *Ulex*, the two closely migrating bands have molecular weights of 43,000 and 45,000. The subunit molecular weights of this lectin have not been reported previously, but the molecular weight of the intact molecule is approximately 160,000⁹. Thus, the *Ulex* lectin may be a tetramer. Commercially available WGA and concanavalin A (Miles) were identical by SDS gel analysis with our purified products. The agglutination activity per mg protein of these commercial lectins was equal to or slightly less than those prepared in our laboratory.

These findings suggest that formalinised erythrocytes can be used as a general affinity adsorbant for the purification of lectins from many sources. The simplicity of the method and the high yields and purity of the product recommend it for general use.

TABLE 1 Purification of four lectins by adsorption to formalinised erythrocytes

Source	mg Crude protein added to suspension	FE type	FE (packed ml)	Eluant	mg Recovered	% Lectin recovered*
Jack bean meal	9	Chicken	3	α -Methyl-D-mannopyranoside	1.7	57
Wheat germ lipase	84	Human Type A ₁	3	N-Acetyl-D-glucosamine	2.9	60
<i>Ulex europaeus</i> seeds	64	Human Type O	3	L-Fucose	1.8	50
Lima beans (<i>Carolina sieva</i>)	300	Human Type A ₁	5	N-Acetyl-D-galactosamine	2.0	100

FE, formalinised erythrocytes. Crude extracts from jack bean meal (Sigma) and wheat germ lipase (Miles) were partially purified according to published procedures¹¹, whereas crude extracts of *Ulex europaeus* seeds (F. W. Schumacher Co., Sandwich, Massachusetts), and lima beans (W. Atlee Burpee Co., Clinton, Iowa) were used directly. The jack bean meal was extracted in 0.15 M NaCl. The extract was concentrated with (NH₄)₂SO₄ and dialysed against 0.15 M NaCl. The product was adsorbed to formalinised erythrocytes. The wheat germ lipase was suspended in water, heated for 10 min at 58°C, chilled to 4°C, centrifuged at 10,000g for 15 min and the supernatant filtered, concentrated by ultrafiltration and made up to final concentration of 0.25 M NaCl, 0.05 M sodium phosphate, pH 7.0 before formalinised erythrocytes were added for adsorption. *Ulex europaeus* seeds and lima beans (100 g of each) were ground in a coffee grinder and extracted in 1.5 l 0.15 M NaCl for 15 h at 4°C with gentle shaking. The resulting suspension was filtered through cheesecloth, centrifuged at 10,000g for 30 min, concentrated by ultrafiltration and adsorbed with formalinised erythrocytes.

* % of lectin activity present in the initial extract which was recovered in the final product.

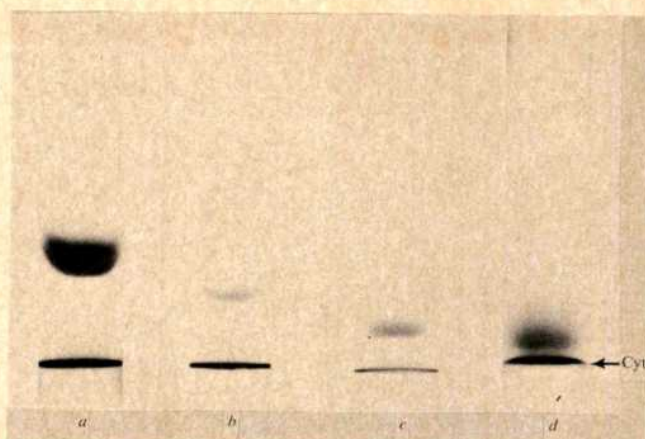


FIG. 1 Polyacrylamide gel electrophoresis of purified lectins. Lectins (100 to 300 μ g) were dissolved in 0.1% SDS, 0.01 M β -mercaptoethanol, applied to 10 cm polyacrylamide gels (2.5% stacking gel, 7.5% running gel) and electrophoresed in 0.1% SDS, 25 mM Tris, 192 mM glycine, pH 8.4. The gels were stained with Coomassie blue¹² and molecular weights estimated by comparison with protein standards¹³. Cytochrome c (Cyt) was added to all samples. a, *Ulex* agglutinin; b, lima bean agglutinin; c, concanavalin A; d, wheat germ agglutinin.

In a search of the literature for a method which might be similar to ours we found that Avrameas and Guilbert¹⁰ used glutaraldehyde-fixed erythrocyte stromata as affinity adsorbents for lectins. Since neither the binding capacity of this adsorbent for lectins nor the electrophoretic purity of the products are described, it is difficult to compare their method with that described here.

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Autoradiography of intravenously injected ¹⁴C-nicotine indicates long-term retention in the respiratory tract.

INVESTIGATIONS using autoradiography¹ to monitor the distribution in mice of ¹⁴C-labelled nicotine administered intravenously showed a consistently high concentration in the entire respiratory tract. A number of mice, some of them pregnant, were each given a single intravenous dose and then were autoradiographed at postinjection times varying between 5 min and 30 d. The injected dose was 0.4 μ Ci per g body weight (corresponding to 1.79 μ g nicotine per g body weight) for the non-pregnant mice, and 0.25 μ Ci per g body



FIG. 1 Details of autoradiograms of mice, a, 24 h and, b, 30 d after intravenous injection of ¹⁴C-nicotine. Note the marked accumulation and retention in the bronchi (white areas).

weight (corresponding to 1.12 μg nicotine per g body weight) for the pregnant mice. The nicotine used was labelled in the methyl group (The Radiochemical Centre, Amersham, England, specific activity 73 μCi per mg^{-1}). Autoradiograms of whole body sagittal sections of the mice were made as described earlier¹. The times of exposure varied from 2 to 4 weeks.

At 5 min there were many sites of localisation in addition to the respiratory tract, such as the central nervous system (CNS), ganglia, and the adrenal glands which have previously been reported by our coworkers^{2,3}. After 1 h and later, the respiratory tract was increasingly dominant in the distribution picture as the radioactivity disappeared from the other areas. The main site of accumulation seemed to be the respiratory mucosal lining: the nasal mucosa, larynx, trachea and bronchi (Fig. 1a). The same pattern persisted after 30 d, although the concentration in the bronchi had now decreased it was still significant (Fig. 1b).

Most other tissues were rather rapidly cleared of the radioactive substance. After 30 d, however, there was still a low level present in the skeletal muscles and in the myocardium. Radioactive material was also observable in the urinary bladder. Although less persistent, a high uptake and retention could also be seen in the oesophageal mucosa.

In the pigmented animals a strong and persistent accumulation occurred in the tissues containing melanin. This has earlier been observed for many polycyclic drugs and seems (providing the dose exceeds a certain critical level) to cause damage of the tissues involved, for example, the skin, eye, inner ear and substantia nigra of the brain^{4,5}.

Long term accumulation in the respiratory tract, however, has not been previously observed in our autoradiographic studies of various types of chemicals. In full term fetuses, a selective accumulation could be seen in the respiratory tract (larynx, trachea and bronchi) and in the melanin bearing tissues.

Further investigations are needed to establish the chemical structure of the labelled compound which accumulates in the respiratory tract. To judge from the rapidity with which the uptake proceeds, it seems likely that the nicotine is trapped as such, but may later be metabolised. The mechanism of accumulation also needs further investigation. Affinity for some endogenous substance in the respiratory mucosal cells is one possibility.

If the observed accumulatory mechanism is also present in humans, smoking may lead to a considerable retention of some nicotine derivative in the respiratory mucosa. Oddly enough, an accumulation should also be expected if nicotine is administered by other routes than by inhalation (such as the use of snuff, chewing tobacco and nicotine tablets). It might, therefore, be worthwhile to look into the possible role of this accumulatory mechanism with respect to the pathogenesis of respiratory cancer and of bronchitis.

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Effect of thiocyanate on nitrosation of amines

CARCINOGENIC nitrosamines are formed by chemical reaction between nitrous acid and secondary and some tertiary amines in the stomachs of rodents¹⁻³. Nitrosamines could be formed similarly in the human stomach and so present a carcinogenic hazard to man. The nitrosation reaction is catalysed by some anions⁴ particularly thiocyanate^{5,6}. Thiocyanate is secreted in saliva which in non-smokers normally contains about 50 mg l^{-1} (approximately 1 mM) but in smokers contains three to four times this concentration⁷. In the absence of catalyst the nitrosation of N-methylaniline and some other secondary amines is maximal at pH 3, but in the presence of thiocyanate the reaction proceeds much more rapidly in acid conditions, such as in gastric juice, between pH 1 and 2 (Fig. 1). At pH 1.5, 1.0 mM thiocyanate increases the rate of reaction about 550 times. Under these acid conditions below pH 2 the nitrosation reaction rate is proportional to the concentrations of (1), nitrite; (2), thiocyanate; and (3), amine⁸. At pH 3.5 and greater thiocyanate has less catalytic action and the predominant reaction is proportional to the square of the nitrite concentration, but is slow.

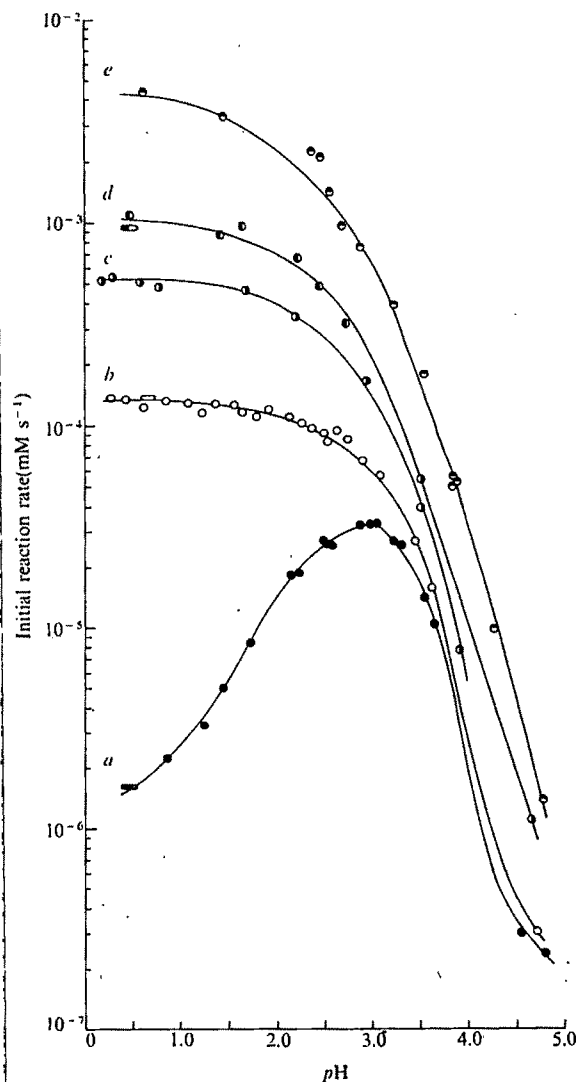


FIG. 1 Initial rate of reaction of N-methylaniline (0.1 mM) and sodium nitrite (0.1 mM) with various concentrations of sodium thiocyanate against pH at 25° C. a, thiocyanate absent; b, 0.025 mM thiocyanate; c, 0.1 mM thiocyanate; d, 0.25 mM thiocyanate; e, 1.0 mM thiocyanate.

The volume of saliva secreted by man is about 1.5 l d⁻¹ and that of gastric juice between 2 and 3 l d⁻¹. If the daily intake of water in food and drink were 3 l d⁻¹ then saliva would be diluted some five-fold in the stomach. The thiocyanate concentration in the stomach could therefore be about 0.2 mM in non-smokers and 0.6 mM in smokers; these concentrations of thiocyanate would increase the nitrosation reaction 100 and 300 times at pH 2. Saliva also contains up to 200 mg l⁻¹ nitrate^{9,10} which is probably derived from food, water and tobacco smoke. Some of the nitrate is reduced to nitrite in the mouth. The smoke from a cigarette contains oxides of nitrogen which might produce 0.5 mg of nitrite in the body so that 20 cigarettes could yield 10 mg nitrite. This amount of nitrite could also be present in 200 g of ham or Frankfurter sausages.

Cigarette smoke contains cyanide compounds which are converted to thiocyanate in the body by the action of rhodanese (thiosulphate-sulphur transferase). The smoke from a cigarette contain up to 0.5 mg cyanides which could yield double this amount of thiocyanate. The daily output of saliva of smokers, however, contains some 200 mg more thiocyanate than that of non-smokers. Thiocyanate is secreted both by the kidney and salivary glands and the volumes of urine and saliva are about the same. Smoking generally increases the flow of saliva¹¹.

Non-smokers excrete three times more thiocyanate in urine than in saliva, that which is secreted in saliva being reabsorbed from the alimentary tract. In smokers the urinary and salivary thiocyanate excretions are about equal. Thus smoking seems to change the route of thiocyanate excretion either by inhibition of kidney secretion or stimulation of salivary secretion. Although cigarette smoke inhibits the activity of rhodanese of guinea pig tissues it increases the thiocyanate concentration of the salivary glands, lung, kidney and brain¹². This is because rhodanese activities are high and smoke contains cyanide compounds.

Nitrosamines are formed in cigarette smoke by the reaction of oxides of nitrogen with secondary and tertiary amines. The most abundant nitrosamine in cigarette smoke seems to be nitrosornicotine¹³ which is carcinogenic¹⁴ and formed from nicotine. The formation of nitrosornicotine would be greater in the more acid smoke of British cigarettes than in the less acid smoke of other cigarettes, cigars or pipes. The synthesis of nitrosamines in the stomach is probably greater in smokers than non-smokers because of the hydrocyanic acid and oxides of nitrogen present in tobacco smoke. Reduction in the concentration of oxides of nitrogen in tobacco smoke should reduce the amount of nitrosamines formed by the smoke itself and in the stomach.

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Thymidylate concentration in megaloblastic anaemia

MEGALOBlastic anaemia is thought to be caused by a disturbance of DNA synthesis in the bone marrow. The most common causes are deficiencies of folate or vitamin B₁₂ which are thought to interfere directly or indirectly with supply of thymidine triphosphate (dTTP, thymidylate) one of the four immediate precursors of DNA^{1,2} (Fig. 1). We have previously shown that acute folate deficiency caused by the antifolate drug, methotrexate, does indeed cause a fall in the intracellular concentration of dTTP³. The present paper reports studies of the cellular concentration of dTTP and of the other three deoxyribonucleotide precursors of DNA, deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP) in the bone marrow cells and phytohaemagglutinin (PHA)-stimulated lymphocytes from patients with untreated megaloblastic anaemia due to vitamin B₁₂ or folate deficiency. Surprisingly, no reduction in dTTP concentration was found in either cell.

Bone marrow was obtained from 20 patients with untreated megaloblastic anaemia (16 Addisonian pernicious anaemia, 4 nutritional folate deficiency). Their haemoglobin levels ranged from 3.1 to 9.8 g per 100 ml and all had florid megaloblastic marrow changes and adequate iron stores. Peripheral lymphocytes were obtained from 15 of these patients. Normal control subjects consisted of 70 healthy adult members of the medical and technical staff of the Hammersmith Hospital. Bone marrow samples were obtained from 13 of these normal subjects and from 14 adult patients with untreated acute myeloid leukaemia in relapse (30–90% blast cells in the marrow).

The deoxyribonucleotide concentrations in bone marrow are shown in Table 1. The levels of all four, dATP, dGTP,

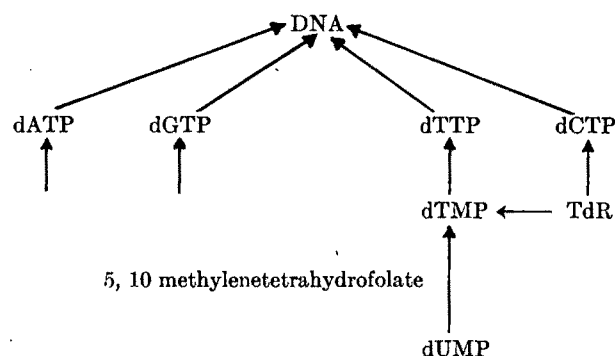


Fig. 1 The synthesis of DNA from four deoxyribonucleoside triphosphates (d, deoxy; A, adenosine; G, guanosine; T, thymidine; C, cytidine; U, uridine; TP, triphosphate; MP, monophosphate; TdR, thymidine).

TABLE 1 Deoxyribonucleoside triphosphate pools in human marrow cells

Diagnosis	dATP			dGTP			dTTP			dCTP		
	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range
Normal	13	1.5	0.2-3.0	10	0.4	0.1-0.6	14	1.4	0.5-2.2	14	0.6	0.1-1.0
Acute myeloblastic leukaemia	14	3.1	0.2-9.3	14	1.5	0.3-4.9	18	5.8	0.6-22	15	1.2	0.3-4.9
Megaloblastic anaemia	17	3.3	0.7-13.6	11	2.2	0.1-5.7	20	5.4	0.8-23.7	9	2.0	0.3-4.6

Bone marrow cells were collected into 5 ml ice-cold Hanks' balanced salt solution containing 500 units sodium heparin. After centrifugation, mature red cells were haemolysed in distilled water for 30 s before restoration of isotonicity with 0.6 M KCl. The cells were washed once in ice-cold phosphate-buffered saline. Extraction of the deoxyribonucleotides was performed by adding 1 ml 60% methanol, cooled to -20°C , to the cell pellet⁴. The tubes were then stood overnight at 4°C before removal of the methanol by evaporation. The assay of the deoxyribonucleotides has been described^{5,6}. For estimation of dATP and dTTP, poly(dA-dT) was used as primer while for dGTP and dCTP, DNA was used as primer. Radiochemicals were purchased from Radiochemical Centre, Amersham, poly(dA-dT) and DNA polymerase (*Micrococcus luteus*) from Miles Laboratories and calf thymus DNA from Sigma Laboratories.

Results are expressed as pmol per 10^6 cells.

dTTP, and dCTP, were greater in megaloblastic than normal marrow cells. It is known, however, that proliferating cells, in general, show greater deoxyribonucleotide concentrations than resting cells⁷. Since megaloblastic marrows contain a greater proportion of primitive cells than normal, the levels of the DNA precursors are also compared with those in the bone marrows of patients with untreated myeloblastic leukaemia which also contain a high proportion of primitive (albeit abnormal) cells. There was a wide variation in individual results, but there was no significant difference between the mean dTTP concentration in the marrow cells of patients with untreated megaloblastic anaemia and acute myeloblastic leukaemia. The ratio of dTTP concentration to that of dATP, dGTP or dCTP, was also similar in these two diseases.

The mean deoxyribonucleotide concentrations in PHA-stimulated lymphocytes from patients with megaloblastic anaemia were not significantly different from normal at either 48 h or 72 h of culture (Table 2). In particular, the dTTP concentrations were similar in megaloblastic and normal lymphocytes. In neither bone marrow nor peripheral lymphocytes were the deoxyribonucleotide concentrations different between the folate-deficient and vitamin B₁₂-deficient megaloblastic cells (not shown).

These results show that the free concentrations of the immediate precursors of DNA, the four deoxyribonucleoside triphosphates, are normal in the PHA-transformed lymphocytes of patients with untreated megaloblastic anaemia. The concentrations of these four precursors are increased in megaloblastic bone marrow compared with normal bone marrow, but only in keeping with the increased proportion of primitive cells in megaloblastic marrow compared with normal. No selective reduction of thymidine triphosphate (dTTP) concentration could be demonstrated in either cell system as might have been expected both on the basis of previous observations of a block in thymidylate synthesis in bone marrow and in PHA-stimulated lymphocytes of patients with untreated megaloblastic anaemia^{1,2} and on the basis of more recent observations of a dramatic decrease in cell dTTP concentration produced by methotrexate *in vitro*³.

Various theories may be proposed to explain the rather surprising finding of a normal cell thymidylate concentration

in megaloblastic anaemia. It seems most likely to us that compensation has occurred in the cells of patients with chronic folate or vitamin B₁₂ deficiency to maintain normal cell dTTP concentrations despite defective synthesis of thymidylate from deoxyuridylate (Fig. 1). This compensation could take the form of increased salvage of preformed thymidine from degradation of DNA in the bone marrow and in other tissues. The activity of the enzyme, thymidine kinase, which is necessary for the salvage of preformed thymidine is indeed increased in the bone marrow cells and PHA-transformed lymphocytes in untreated megaloblastic anaemia^{9,10}. Moreover, intramedullary haemolysis would lead to excessive release of preformed thymidine in the marrow in megaloblastic anaemia. Slow DNA synthesis, known to be a feature of megaloblasts¹¹, could also contribute to maintenance of the dTTP concentration by reducing utilisation of dTTP.

Some other explanations for the normal dTTP findings are possible. It could be that the present finding of normal dTTP concentrations in megaloblastic anaemia is artefactual as a result of replenishment of the dTTP concentration *in vitro* in our cultures but this is unlikely. Though the medium, TC 199, used for culture of lymphocytes does contain thymine, 10^{-6} M, culture in a specially prepared thymine-free TC 199 made no difference to the measured dTTP concentration in PHA-stimulated megaloblastic lymphocytes from three patients (unpublished observations). Further, the bone marrow dTTP estimations were made on marrow samples cooled in Hanks' solution (free of thymine or thymidine) and extracted immediately after aspiration. Another potential source of artefact is that a compound which accumulates in megaloblastic cells other than dTTP might be measured as dTTP by the technique used here. We have no evidence to support or refute this possibility, though in view of our finding of a consistent decrease in measured dTTP concentration with methotrexate *in vitro*³, we feel it is unlikely. A further possibility is that an overall normal cellular dTTP concentration in megaloblastic anaemia may mask an intracellular redistribution of this compound with a reduction in nuclear dTTP masked by a increase in the extranuclear concentration of this compound. Hydroxyurea, which inhibits DNA synthesis mainly by antagonism of ribonucleotide reductase, has also been suggested to damage the nuclear mem-

TABLE 2 Deoxyribonucleoside triphosphate concentrations in human PHA-stimulated lymphocytes

Cells	Time of culture	dATP			dGTP			dTTP			dCTP		
		No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
Normal	48 h	10	1.9-6.1	3.6	2	0.6-2.4	1.5	11	3.2-13.4	8.7	2	1.0-3.1	2.0
Megaloblastic anaemia	48 h	5	1.2-5.7	2.7	—	—	—	5	3.7-11.4	7.6	—	—	—
Normal	72 h	69	0.7-8.0	3.7	27	0.5-5.6	1.9	70	1.1-17.7	9.4	29	0.8-6.3	2.9
Megaloblastic anaemia	72 h	15	2.5-6.5	4.2	4	1.2-5.1	3.1	15	3.1-16.3	10.8	4	3.8-4.7	4.4

Lymphocyte cultures were set up in autologous serum and TC 199 as described⁸ except Triosil-Ficoll was used instead of iron filings to remove neutrophils. Cultures of 3×10^6 cells were made in 1 ml autologous serum and 2 ml TC 199 (Biyroughs-Wellcome) and 0.1 ml PHA. After 48 or 72 h, the cells were washed twice in ice-cold phosphate-buffered saline.

Results are expressed as pmol per 10^6 cells

brane, and loss of deoxyribonucleotides from the nucleus may play a part in its action¹². Further studies of the sub-cellular localisation of dTTP in megaloblasts are, therefore, necessary. Finally, there has been discussion whether the mono or diphosphate rather than the triphosphate derivatives of the deoxyribonucleosides are the immediate DNA precursors. We have not measured the cell concentration of thymidine mono or diphosphate in megaloblastic anaemia but recent evidence does firmly support the deoxyribonucleoside triphosphates as the crucial precursors¹³.

In a previous study⁸ we found that acute folate deprivation caused by methotrexate *in vitro* in PHA-stimulated lymphocytes caused an increase in dATP concentration as well as a decrease in dTTP. The present results show that in chronic megaloblastic anaemia there is no increase in dATP but that the dATP concentration parallels that of the other deoxyribonucleotide triphosphates. Compensations must have occurred to balance the deoxyribonucleotide pools at a slower rate of DNA synthesis in the chronic state.

Whatever the explanation for the normal deoxyribonucleotide concentrations in untreated megaloblastic anaemia, our results provide further information on the disturbance of DNA synthesis in this disorder and complement previous observations that the base composition of the DNA itself is normal in the bone marrow in untreated megaloblastic anaemia¹⁴. They are consistent with the view that slowed DNA synthesis in some way gives rise to the characteristic morphology and intramedullary cell death of megaloblastic anaemia.

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Catecholamine transport through a lipid barrier

NORADRENALINE and adrenaline are stored in relatively high concentration together with nucleotides (mainly adenosine-5'-triphosphate (ATP)), bivalent cations (for example Ca^{2+} and Mg^{2+}) and water-soluble proteins, in special intracellular organelles such as chromaffin granules of adrenal medulla¹. In the uptake of catecholamines by these organelles a specific mechanism is probably involved at the level of the granular membrane². Furthermore, formation of low molecular weight complexes between catecholamines and ATP^{3,4} and mixed amine-nucleotide aggregates of apparent high molecular weight⁵⁻⁸ has been demonstrated. The present experiments deal with the role of these interactions for the uptake and storage of the amines. Evidence will be presented that an 'uphill' transport of neurotransmitters occurs if ATP is present on one side of an artificial lipid barrier and that this effect is connected with a reduction in the osmotic pressure of the biogenic monoamines by ATP.

Permeation studies were carried out at 37° C with commercially available equipment (resorption model of Stricker, SM 16750, Sartorius-Membranfilter GmbH, Göttingen), consisting of a diffusion cell with two compartments, A and B, of equal size (100 ml) separated by an artificial lipid barrier, and a peristaltic pump for the circulation of the aqueous solutions along the lipid barrier. The latter, which had an effective size of 80 cm² and a thickness of about 100 µm, was prepared by soaking a membrane filter (type RS, Sartorius, Göttingen) at 30° C with a mixture of 97.7% lauryl alcohol and 2.3% diethyl succinate. (–)-Noradrenaline (0.0095 mol l⁻¹) (purum, Fluka, Buchs) or 0.0055 mol l⁻¹ (–)-adrenaline (crystalline, Merck, Darmstadt) or 0.0083 mol l⁻¹ β-phenethylamine (Sigma, St Louis) were dissolved together with 0.024 mol l⁻¹ CaCl_2 in K-Na phosphate buffer of pH 5.8 (molarity: 0.067). ATP (0.10 mol l⁻¹) was added to the solution in compartment B, but not to A. The pH of the

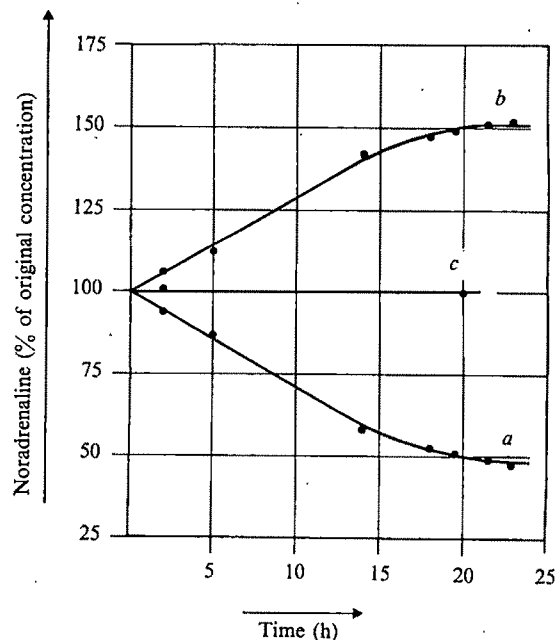


FIG. 1 Transport of noradrenaline through a lipid membrane from compartment A to compartment B at 37° C. Both compartments contain 0.0059 mol l⁻¹ noradrenaline at the beginning of the experiment. Compartment B also contains 0.10 mol l⁻¹ ATP. The pH in both compartments is adjusted to 5.8 and remains constant during the experiment. 100%, Noradrenaline concentration at the beginning of the experiment; a, compartment A; b, compartment B; c, compartments A and B in the absence of ATP in compartment B. Results are the averages of four experiments.

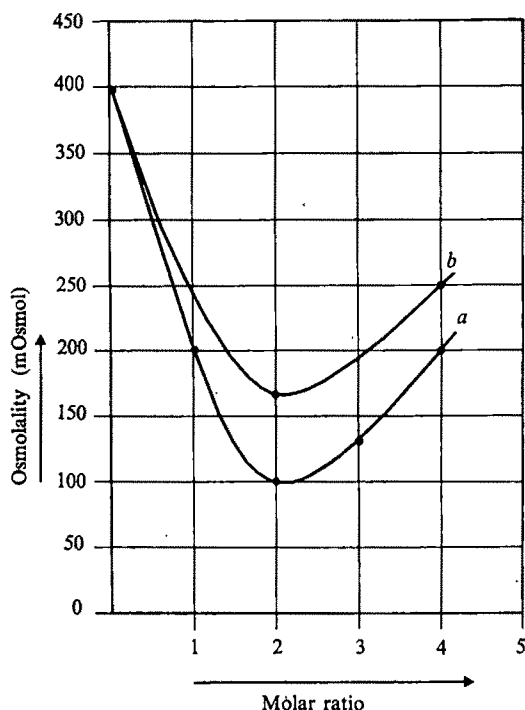


Fig. 2 Effect of added catecholamines on osmolality of an aqueous solution of 0.19 mol l⁻¹ ATP, containing CaCl₂ in a molar ratio to ATP of 0.24 to 1, at 37° C. a, Catecholamine = noradrenaline; b, catecholamine = adrenaline. Results are average of three experiments.

solutions was readjusted to 5.8 with 2 N NaOH. Auto-oxidation of noradrenaline was prevented by adding 0.01% ascorbic acid (Hoffmann-La Roche, Basel) and by bubbling nitrogen through the solutions during the course of the experiments. The concentrations of noradrenaline⁹ and of ATP¹⁰ were checked by fluorescence spectroscopy, those of adrenaline and β -phenethylamine by ultraviolet spectroscopy. Analysis by fluorescence spectroscopy showed that the artificial lipid barrier was impermeable to ATP, corresponding in this respect to membranes of amine storage organelles¹¹.

Osmolalities were determined by isothermal distillation¹² at 37° C using aqueous NaCl as reference solutions. The osmolalities of all solutions were estimated by measurement of freezing point depression with a semi-micro osmometer (Knaur, Berlin). Controls of the osmotic pressure of the solutions of inorganic ions by isothermal distillation at 37° C showed that their osmolalities were practically independent of temperature. The osmotic pressure due to the inorganic ions was subtracted, so that the results presented in the graph corresponded to ATP (0.19 mol l⁻¹) and its mixtures with the catecholamines only. The pH of the solutions containing noradrenaline (0.76 mol l⁻¹) in a molar ratio to ATP of 4 to 1 was 5.8, that of the solutions of lower molar ratios or with ATP only correspondingly lower (0.6). Control experiments with adjustment of the pH of the ATP solution to 5.8 (using 2 N NaOH) in the absence of noradrenaline gave evidence that the pH effect on osmolality was negligible.

Figure 1 shows the 'uphill' transport of noradrenaline at 37° C. The amine content was the same in both compartments at the beginning of the experiment (0.0059 mol l⁻¹), but compartment B also contained 0.10 mol l⁻¹ ATP. During the experiment, the noradrenaline concentration in compartment A decreased markedly (curve a), and correspondingly an increase occurred in compartment B (curve b). Adrenaline behaved similarly to noradrenaline. No change in catecholamine concentration was to be seen in the absence of ATP from compartment B (curve c). Transport of noradrenaline into the ATP-containing compartment also occurred at higher

initial concentrations of the amine on both sides of the lipid barrier.

Figure 2 demonstrates the effect of the addition of noradrenaline and adrenaline on the osmolality of 0.19 molar aqueous solution of ATP at 37° C. The solutions also contained CaCl₂ in a molar ratio to ATP of 0.24 to 1. The results indicate that mixtures of ATP and noradrenaline and of ATP with adrenaline have substantially lower osmotic pressures than ATP alone. The fraction of catecholamines present in the dissociated state in these mixtures was about 10–20% (based on calculations of apparent average molecular weights obtained by isothermal distillation, sedimentation equilibrium and velocity experiments). In contrast, noradrenaline hydrochloride was dissociated to about 70–80% in the same range of concentrations (determined by isothermal distillation at 37° C). Minimum osmotic pressure existed at a molar ratio of 2:1, catecholamines to ATP, the osmotic pressure of ATP being reduced by a factor of 4 and 2.5 by the addition of noradrenaline and adrenaline respectively. On increasing the molar ratio to greater than 2:1, the osmotic pressure rose again, but even at a ratio 4:1 it was still lower than that of ATP alone.

The accumulation of catecholamines in the ATP-containing compartment B was probably due to intermolecular bonding between the amines and the nucleotide, which is confirmed by our osmolality measurements. Because of the interaction of catecholamines and ATP, the concentration of free catecholamines available for diffusion is likely to decrease in the ATP-containing compartment B, giving rise to a concentration gradient between compartments A and B of the free (diffusible) amine. The catecholamines would then penetrate by diffusion from compartment A into compartment B, where most of them are bound to ATP. The overall displacement of catecholamines from A into B probably stops when the concentration of the free catecholamines in the two compartments is the same. This view is supported by the finding that β -phenethylamine, which does not accumulate in the ATP-containing compartment of the diffusion chamber, did not display marked aggregation with ATP in the sedimentation equilibrium and velocity experiments (our unpublished observations).

The present results with an artificial lipid membrane show that in the presence of ATP and bivalent cations, catecholamines can be accumulated against a concentration gradient without a specific, energy-dependent transport mechanism being present. This finding confirms the previous hypothesis by which intragranular formation of aggregates between amines, nucleotides, bivalent cations and possibly proteins is an essential mechanism for the uptake and storage of biogenic amines by adrenal chromaffin granules and probably by other storage organelles, blood platelets for example¹³. A tentative structure for the intragranular organisation of catecholamines and ATP has recently been proposed¹⁴.

In conclusion, our experiments provide direct evidence that transport and accumulation of catecholamines can occur as a consequence of their aggregation with ATP. This aggregation may considerably increase the efficiency of the granular membrane pump in storing biogenic amines.

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The contribution of activation processes to the length-tension relation of cardiac muscle

It is generally agreed that the form of the length-tension relation of a fully activated skeletal muscle is determined by the overlap of actin and myosin filaments within the sarcomere. Figure 1 shows the length-tension curve for tetanised frog skeletal muscle¹, which is thought to be fully activated at lengths above 75% L_{max} (ref. 2). This is the form of the curve that would be expected from any muscle of similar sarcomere structure to frog muscle, provided that it has a constant degree of activation (either complete or partial) at muscle lengths above 75% L_{max} . The sarcomere structure of cardiac muscle is similar to that of frog muscle³, yet the shape of its length-tension curve is strikingly different (Fig. 1). In skeletal muscle deviation from the 'ideal' curve shown in Fig. 1 has been attributed to variation in the degree of activation when the muscle is stimulated at different lengths. We have investigated this possibility in cardiac muscle by studying the effect on the length-tension

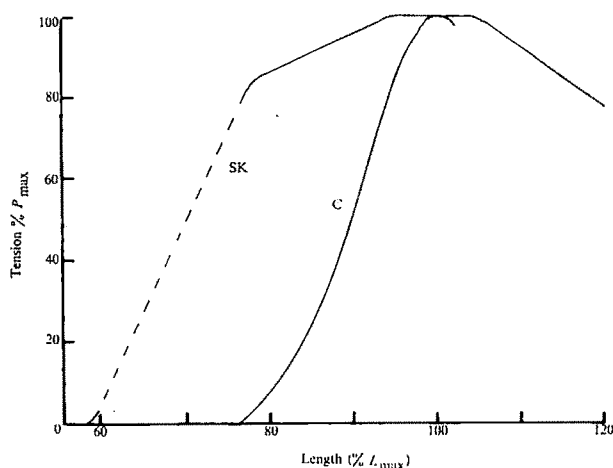


Fig. 1 The length-tension relationships for skeletal (Sk) and cardiac (C) muscle. The ordinate is the developed tension as a percentage of the maximum developed tension (P_{max}) and the abscissa is the length as a percentage of the length (L_{max}) at which developed tension is maximum. There is evidence that the sarcomere spacings at L_{max} are similar in frog skeletal and cat papillary muscle³. The dashed line on the skeletal muscle curve is the region over which activation may be incomplete².

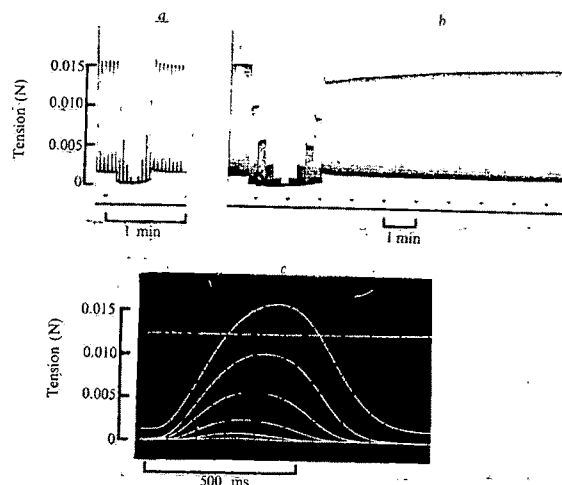


Fig. 2 Experimental records of twitch responses of a cat papillary muscle (L_{max} 3.3 mm, diameter 0.6 mm) to electrical stimulation at 20 min⁻¹. *a*, Experimental sequence used to obtain the length-tension curves shown in Fig. 3. The length of the muscle is first decreased and then increased again in five equal steps of 0.165 mm (5% L_{max}). Tension is measured in newtons. *b*, This shows an alternative sequence in which the muscle was held at each length for five beats; the difference between the first beat after a change of length and subsequent beats is attributed to the presence of a series viscoelastic element in the preparation. Note that tension production takes several minutes to recover to its previous steady level after the muscle has been returned to its original length. *c*, High speed (oscilloscope) records of the twitch responses during the descending part of the tracing shown in *a*.

relation of varying the degree of activation produced by electrical stimulation: we have done this by altering the concentration of calcium in the bathing medium and by using paired pulse stimulation⁴.

The experimental procedure was as follows. An isolated papillary muscle from the right ventricle of a kitten was stimulated electrically at 30° C to give isometric twitch contractions every 3 s (see ref. 5 for further details about technique). The length-tension curve was determined as quickly as possible by changing the length of the muscle between successive beats (Fig. 2*a*), starting near L_{max} and working down to a length at which no increase in tension was detectable on stimulation and then returning in similar steps almost to L_{max} . This procedure avoided the complications introduced by the slow changes in tension development that occur when the muscle is allowed to contract for a longer period at short lengths (compare Fig. 2*b* and *a*). The value of L_{max} was then estimated by stretching the muscle in smaller steps until the developed tension (peak tension minus resting tension) started to fall. The entire procedure was performed when the calcium concentration in the bathing solution was 2.0 mM, 0.5 mM, 8.0 mM and then repeated at 2.0 mM.

The resting tension and peak tension were measured at each length and the values obtained on the ascending and descending limbs of the cycle were averaged to minimise the influence of a viscous element or other factors⁵ which might make the first beat at lengths below L_{max} different from subsequent ones (see Fig. 2*b*). The results have been plotted in two ways, as in Fig. 3*a* and *b*, which show the data obtained from a representative muscle. In Fig. 3*a* the peak tension and resting tension are given in N mm⁻² as a function of the actual muscle (mm), and the estimated value of L_{max} is marked on each curve. The length-tension relation for the resting muscle is unaffected by changes in the calcium concentration, but the curve for peak tension is affected in several ways: there is a very slight shift of L_{max} to longer muscle lengths when the calcium concen-

tration is raised, but the main effect is a large potentiation of tension development, which is proportionally greatest at short muscle lengths. This is clearly seen in Fig. 3a, but it becomes much more obvious when the data are replotted so that the maximum tension developed at each calcium level is normalised to 100% and muscle lengths are expressed as a percentage of L_{max} (Fig. 3b). If the normalised curves are not superimposable at lengths other than L_{max} , then the intervention must have altered the degree of activation relatively more at these lengths than at L_{max} : the implication of this would be that activation processes in the muscle are length-dependent. Thus the conclusion from the results in Fig. 3b is that the form of the length-tension relation of cardiac muscle depends at least in part on a variation in the degree of activation with length. The 2 mM- Ca^{2+} data shown in Fig. 3 (and comparable data from three similar experiments) suggest that the form of the length-tension relation changes slightly with time, but any such effect is too small to affect our conclusion.

Since the 8.0 mM curve is shifted towards that expected from the sliding filament theory, one wonders if by increasing the activation further a curve which approximated more closely to the sliding filament theory could be obtained. To this end we have increased the degree of activation by paired pulsing which in some circumstances may produce tensions close to the maximum of which the muscle is capable at that length⁴. This altered the shape of the length-tension relation, but only to approximately the same extent as 8.0 mM calcium.

One possible objection to our results is that peak tension is not a satisfactory index of contractility if the time to peak tension is altered by the intervention being investigated (see Fig. 2c)⁶. However, the length-tension relation for $(dP/dt)_{max}$, an index of contractility that is unaffected by changes in time to peak tension, varied with the calcium level in just the same way as the length-tension relation for developed tension (Fig. 3b). The argument that tension changes in parallel elastic structures might have interfered in some way with our measurements can also be dismissed because the difference we have noted in the form of the length-tension relation is present at lengths where there is no resting tension (Fig. 3a). We have also considered the possibility that the change seen in the normalised curve could be due to the presence of an internal load that increases in size as the muscle length decreases (such a load must exist because the relaxed length of a muscle is greater than the shortest length to which it can contract). Although

this is a possible explanation, quantitative estimates of the size of the load required to produce the observed shift in the normalised curves are not consistent when two of the curves in Fig. 3b are used to predict the shift in the third one; furthermore, the size of the load required to produce the observed effects decreases rather than increases at shorter muscle lengths.

Thus, it appears from Fig. 1 and the results shown in Fig. 3 that in cardiac muscle, as in frog skeletal muscle under some conditions (such as tetanic contractions at short muscle lengths², twitch contractions at room temperature⁷), the form of the length-tension relation is not determined simply by the properties of the sliding filament system. In both types of muscle length-dependent activation processes appear to have an important influence on the form of the curve, at least at lengths below L_{max} . As cardiac muscle is probably confined to working below L_{max} under physiological conditions because of its large resting elasticity, this is a matter of considerable practical importance, but at this stage the nature of the length-dependence of activation processes can only be a matter of speculation. The fact that peak tension is reached sooner at short muscle lengths (Fig. 2c) suggests that deactivation associated with shortening is occurring⁸, and observation of the muscles operating at the foot of their length-tension curve confirms that they are visibly slack at rest and must therefore shorten appreciably before exerting tension. Incomplete activation due to failure of the inward spread of excitation to centrally-placed myofibrils⁹ seems less likely in cardiac muscle than in skeletal muscle because of the much smaller diameter of the cardiac muscle cell.

It seems that in heart muscle in contrast to skeletal muscle the rise in intracellular Ca^{2+} concentration produced by each action potential is insufficient to give full activation of the contractile system. The degree of activation is thought to be affected by the rate of stimulation and extracellular Ca^{2+} concentration¹⁰, the duration of the action potential¹¹, and the effect of various drugs¹²; and the observations reported here provide evidence that muscle length is another factor of importance in determining the completeness of the excitation-contraction coupling process.

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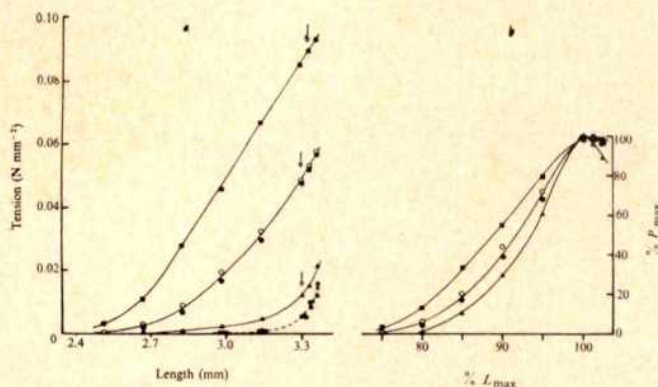


FIG. 3 a, Length-tension curves at three different calcium concentrations (same preparation as Fig. 2) ■, 8 mM Ca^{2+} ; ○, first series at 2.0 mM Ca^{2+} ; ●, repeat series after the determinations at 0.5 and 8 mM Ca^{2+} ; ▲, 0.5 mM Ca^{2+} ; —, peak twitch tension; ---, resting tension. The arrows indicate L_{max} for each curve. b, Normalised length-tension curves at three different calcium concentrations. The ordinate is developed tension as % P_{max} and the abscissa is the length as % L_{max} . Symbols as in a.

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Pattern reconstitution in abdominal segment of *Leucophaea maderae* (Blattaria)

DURING the past few years the cuticular patterns of insect segments has been looked at with increasing interest. Following transplantations of pieces of epidermis¹⁻⁵ all these patterns become remodelled in a characteristic way. Analysis of the patterns led to the idea of a segmentally reiterated gradient. According to this concept any line of a segment with its specific structure is characterised and possibly determined by a specific level of the gradient.

Many of the experiments dealing with the problem of the mutual correlations between the segmental gradient and pattern formation have been performed in the bug *Rhodnius*^{2,6}. In this species the cuticular pattern of the tergites consists of medio-laterally oriented surface ripples. When square pieces of the integument are cut out and rotated through 90° the orientation of cuticular structures in the transplant gradually approaches the normal orientation during the following moults.

There are two main ways this reconstitution of the pattern could occur. (1) It is assumed that the gradient has many of the properties of a concentration-diffusion gradient. A cell differentiates and secretes its specific cuticle according to the level at which it happens to lie. A change of the level of the gradient in the surroundings of the cell is followed by a change of the differentiation properties of the cell; the cell is 'reset' to the new level. Thus the reconstitution of the pattern after a 90° rotation is due to the levelling of discontinuities of the gradient by processes similar to diffusion and the subsequent redifferentiation of the cells. The cells of the transplant remain in their rotated position but change their polarity and pattern. Computer simulations based on these propositions showed agreement with the observed cuticular patterns.⁶ (2) It is assumed that the gradient features and the differentiation properties of the cells are more stable and cannot be changed by diffusion-like processes. The approximation of the pattern towards normality after the 90° rotation could be brought about by a bodily rerotation of the whole transplant. In this case the cells of the transplant would change their position relative to the host tissues but preserve their original differentiation properties. Similar rerotations of grafts had been observed in the eyes of amphibia⁷ and in the legs of cockroaches⁸.

In *Rhodnius* there are no proper epidermal and cuticular markers which could allow a clear decision between the two alternatives. So it seemed desirable to make similar experiments in species better suited for this purpose. This and the following two papers describe rotation experiments performed on the tergites of two hemipteran species and of a cockroach⁵.

Larvae of *Leucophaea maderae* were used for the experiments. The cuticular pattern of the larval tergites consists of two areas: the anterior half of the tergite has a more or less smooth cuticle with only a few tiny bristles; the posterior half has a cuticle with many tubercles of different size in a regular but individually variable distribution. The two fields are separated by a prominent transversal ridge. Square pieces of integument containing the ridge and parts of both regions were cut out and reimplanted after a rotation through 90°. The angle between the ridge of the transplant and a reference line in the host segment was measured on the mounted cuticles of the successive larval instars up to the imago.

As shown in Fig 1a-f the angle between the ridge of the transplant and the reference line diminishes gradually during the following moults. The bristles and tubercles in the central area of the transplant stay in a more or less constant position relative to the ridge (Fig 1g-k). Thus the whole pattern rotates back towards the original position.

The pattern of bristles and tubercles on the tergites of

Leucophaea is highly variable; no two segments have an identical pattern. If it is assumed that after the rotation of the transplant the cells are reset to new gradient levels and according to their new levels generate a new pattern one would expect that the distribution of bristles and tubercles in the newly formed pattern would be different from that in the original. The observation of an identical pattern rotating back towards the original position is a strong indication for a rerotation of the transplant tissues as a whole. The entire pattern changes are not to be explained purely

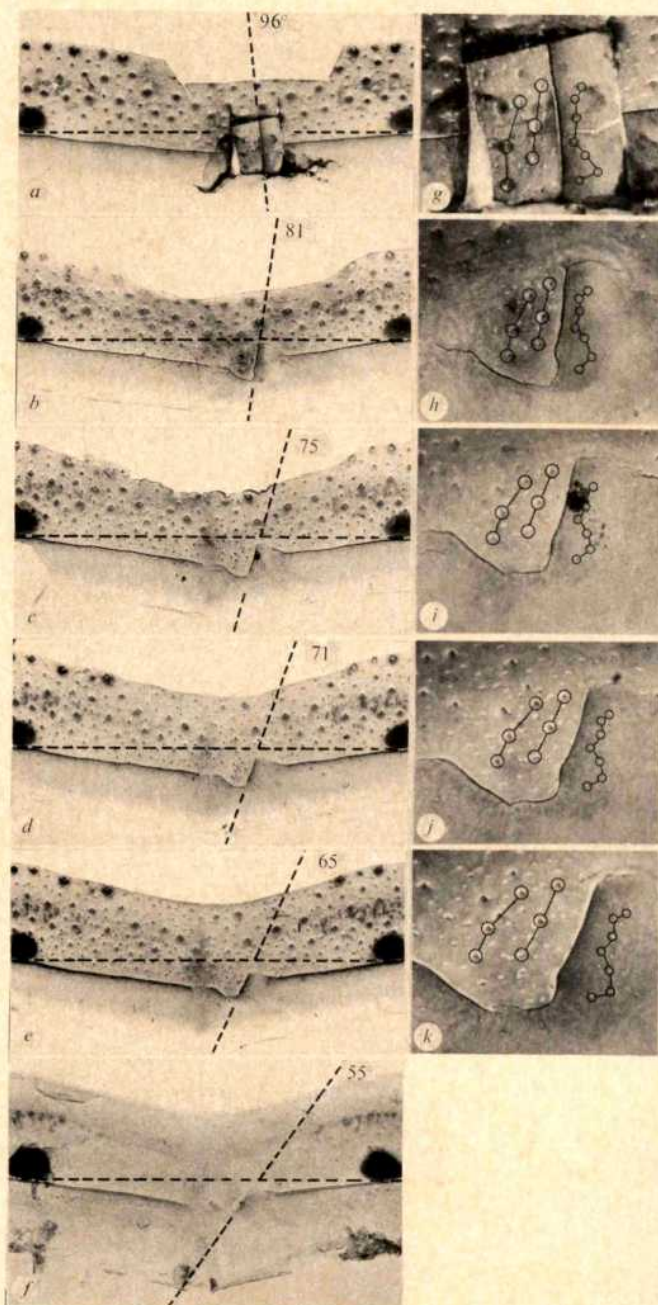


FIG. 1 a-f, Development of an anticlockwise rotated piece of skin during the following moults up to the imago. The cuticle of one instar (between d and e) had been lost. The angle between the ridge of the transplant and a reference line (between insertions of left and right dorso-ventral muscles) diminishes from 96° to 55°. At the right (g-k) the corresponding region of the transplant is shown at a higher magnification. Part of the bristles and tubercles are outlined by circles to demonstrate that the relative positions of ridge, bristles and tubercles of most parts of the transplant remain more or less unchanged; the pattern as a whole rotates back. Magnifications: a, $\times 101$; b, $\times 92$; c, $\times 83$; d, $\times 76$; e, $\times 51$; f, $\times 51$; g-k, $\times 303$.

by rotation however, since this could not explain how the ridge of the transplant joins up with that of the host. The generation of intermediate gradient values by intercalary regeneration is one possible explanation.

Though it is only the pattern on the cuticle whose rotation can be observed in *Leucophaea* there is no doubt that the epidermis of the transplant, too, rotates back since the cuticle is only a secretion product of the epidermis. More direct evidence for the movement of the epidermis is presented in the following contributions^{9,10}. So pattern reconstitution in the tergites of *Leucophaea* is, at least mainly, brought about by a rerotation of the transplant tissues; it is not necessary to assume a resetting of the cells. In this respect the epidermis of the tergites is like the legs in which a resetting to new levels seems to be possible only in proliferating tissues⁵.

The mechanics of the rerotation has not been studied in detail up to now. Muscle contraction as a means for movement can be excluded since the transplant region is completely free of muscles. Most likely the rotation is achieved by an active movement of the border cells, while the more central parts are carried along with them passively as can be judged from the unchanged pattern of large central parts (Fig. 1g-k; see also ref. 10). Pushing the transplant by intercalary regeneration should also be taken into account.

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Cell movement during pattern regulation in *Oncopeltus*

THE cuticle of adult *Rhodnius* (Hemiptera) is marked by mediolaterally oriented surface ripples. From transplantation and rotation experiments on the epidermis Locke¹ demonstrated a segmentally reiterated gradient. We² have proposed a model of the gradient which is considered as a concentration of some diffusible substance, the direction of steepest slope of concentration specifying cellular polarity and thereby the orientation of the ripples. We suggested that the cells might contribute to the stability of the gradient and attempt to maintain their original or 'set' level when transposed.

Rotation of a larval square of epidermis through 90° results in an S-shaped pattern of ripples in the adult cuticle. When such an adult is made to moult again to produce a second cuticle the S-shaped pattern flattens, so that the ripples are now aligned nearer to the mediolateral axis. This change of pattern was associated with the presence of cell divisions in the artificial moult cycle; when the moult cycle was truncated and cell divisions omitted, the first and second

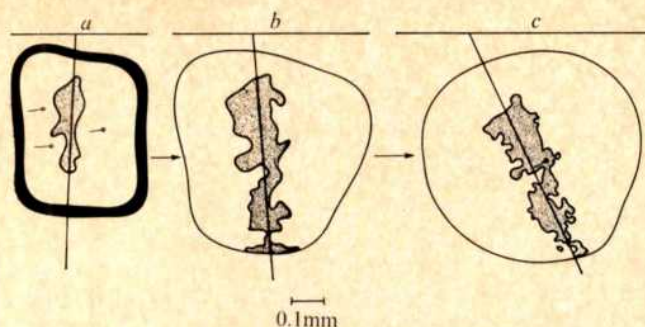


FIG. 1 Camera lucida drawings showing the result of transplanting a piece of orange epidermis containing a transparent clone (shaded) on to a white fourth-stage host. The graft was rotated through 90° clockwise. a, The situation immediately on grafting (clone rotated c. 93°); b, the young fifth-stage (angle of clone now c. 85°); c, the adult (angle of clone now about 65°).

cuticular patterns were almost identical. We therefore suggested that the cells' set level was re-established at some stage in the cell division cycle allowing further diffusion and consequent changed polarity. An alternative cause of pattern change could be bodily rotation of the entire graft in the opposite direction to that of the original experiment⁹. This paper and the two accompanying contributions^{3,4} show that in fact there is bodily rotation of the graft.

Pieces of epidermis were exchanged between wild type (orange pigmented cells) and a mutant, *wb* (white pigmented cells) of *Oncopeltus fasciatus*⁵. The adult hairs and cuticular tubercles were used as polarity indicators, both normally

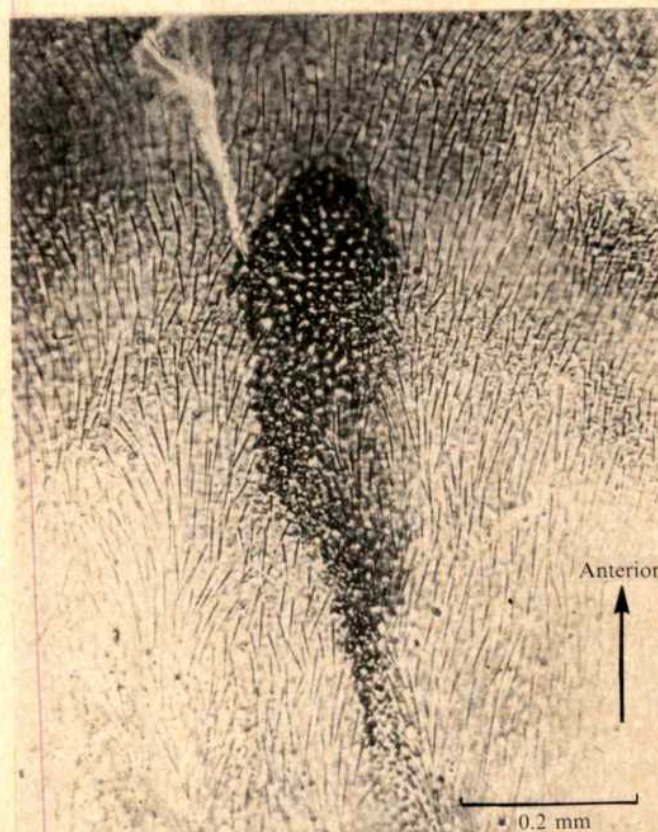


FIG. 2 A strip of orange epidermis (long axis mediolateral) was rotated through 90° clockwise and transplanted on to a white fourth-stage larva. In the adult the strip of orange cells is still clearly visible and still retains the same orientation as it had on grafting (long axis antero-posterior). As in the host, the hairs in the graft point posteriorly.

pointing posteriorly. Following X-irradiation as eggs⁶, fourth-stage larvae were screened for long thin clones which were a different colour than orange and had their long axes parallel to the mediolateral axis of the body. Square pieces of epidermis containing such clones were excised, rotated through 90° and implanted on a white host in the centre of the segment. The graft border and shape and orientation of the clone were drawn by camera lucida in the two succeeding instars (Fig. 1). In some cases the clone was damaged by the operation but in all five cases where the clone survived as a coloured strip, there was clear evidence for the rotation of the clone and therefore for bodily rotation of the graft; in no case did the central hairs become reorientated while the clone remained in the original orientation. In one case the clone itself became S shaped suggesting more cell movement at the edges of the graft.

My unpublished experiments on *Dysdercus fasciatus*, and those of Nübler-Jung⁴ on *D. intermedius* suggest that the rotation of grafts is restricted to a relatively short period (about 20 h⁴) when the epidermis separates from the cuticle; that is, after cell divisions have ceased.

In a second series of experiments (suggested to me by Dr H. Bohn) thin strips of epidermis, with their long axes oriented mediolaterally were taken from orange *Oncopeltus* larvae, rotated through 90° and transplanted on to white larvae. When the graft survived as a strip, it remained oriented close to the anteroposterior axis, but the orientation of the hairs, and the small epidermal tubercles turned so that they pointed posteriorly (Fig. 2).

These two classes of experiments show that the situation is complex; the first shows that cell movement is an important component of pattern regulation, and the second that regulative changes of polarity can occur without bodily rotation of the entire graft. It is still possible that regulation of polarity is achieved by smaller parts of the graft rotating independently. Rotation back of grafts has been demonstrated in other systems (the amphibian ear⁷ and eye⁸, and the insect leg⁹), while polarity changes that are not dependent on rotation are found after juxtapositions of regions from different levels in the axis of both the abdominal segments² and the legs of insects⁹. These changes are associated with cell division.

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Cell migration during pattern reconstitution in the insect segment (*Dysdercus intermedius* Dist., Heteroptera)

If in the insect segment a piece of epidermis is rotated through 90° the changed pattern tends to return to normal after several moults.¹ This observation can be explained by a rotation of the whole transplant (ref. 2 and H. Bohn, personal communication) or by a resetting of the trans-

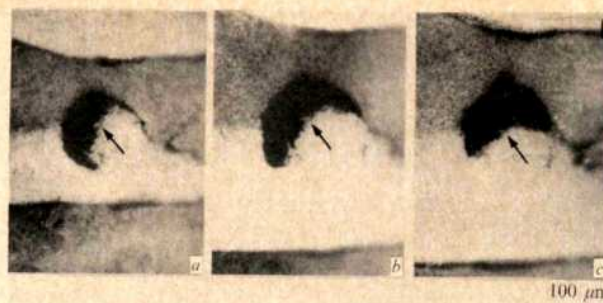


FIG. 1 Development of a transplant rotated anticlockwise through 90° during the third larval instar. *a*, Aspect early in the fourth instar; *b*, late fourth to early fifth instar; *c*, shortly before metamorphosis. Posterior segment border at the bottom. Scale, 100 μm.

posed cells.³ Until now it was not possible to decide between these two mechanisms since pattern reconstitution in the leg or body segment was studied only by the observation of cuticular structures such as bristles and ripples⁴. These structures reflect the physiological status of the epidermal cells only during the short periods of epicuticle formation but not during the intervals in between. Moreover, by studying the cuticle it is rarely possible to determine the exact boundary between host and transplanted epidermis. These disadvantages can be overcome by using a colour mutant⁵ which enables continuous observation of the transplanted cells.

In the wild type of *Dysdercus intermedius* from the third larval instar onwards the anterior zone of the third ventral abdominal segment is red and the posterior zone white. The mutant lacks the red pigment and therefore the anterior zone looks grey. It should be noted that the pigment is located inside the epidermal cells. The cuticle is transparent and permits direct observation of the location and possible movements of the transplanted cells. Transplantations were carried out on third instar larvae as described by Lawrence *et al.*³. The transplants always included the border between the red and white zones. The hosts were of the grey mutant type. The segments carrying transplants were filmed by time-lapse cinematography (1 frame per 3 min) or photographed at daily intervals until after metamorphosis.

Three stages in the development of a transplant which had been rotated anticlockwise through 90° are illustrated in Fig. 1. After the first moult following the operation (Fig. 1*a*) some red cells are found between the white cells of the graft and

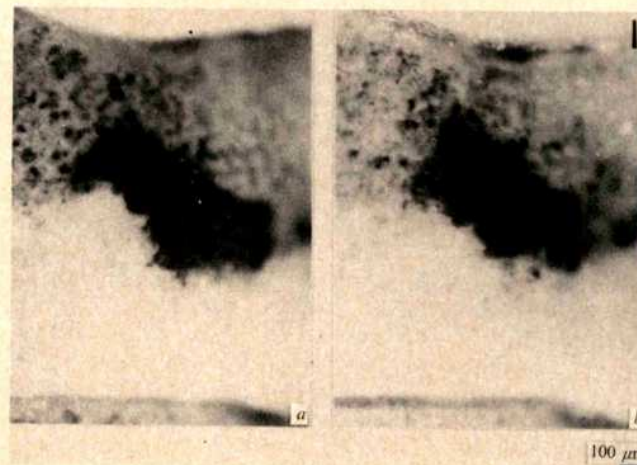


FIG. 2 Time-lapse film which was taken towards the end of the fifth larval instar. *a*, Aspect at the beginning of film (0 h); *b*, at the end (54 h) of the film. Scale, 100 μm.

the grey cells of the host (upper right). This aspect remains stationary for several days while the larva grows and mitoses occur in the epidermis⁶. When the epidermis is detached from the old cuticle in preparation for the next moult the spur at the posterior end of the red patch disappears and the wedge of red cells at the right anterior rim increases considerably in width. This aspect (Fig. 1b) persists through moulting and during the following days. Some days before metamorphosis, when the epidermis again detaches from the cuticle, the posterior region seems to move forward and the wedge at the right is strengthened again. Simultaneously, the angle between the posterior segment margin (at the bottom) and the red/white border within the transplant decreases further. The resulting pattern (Fig. 1c) is retained by the imago. It should be noted that some local peculiarities at the red/white border within the transplant (arrows) persist throughout development. During all these changes no pink cells were observed which might indicate a change of pigment synthesising activities.

The analysis of time-lapse films (Figs 2 and 3) reveals that the rotation of the red/white border with respect to the posterior segment margin and the concomitant changes in shape are accomplished within 20 to 25 h ($28 \pm 1^\circ\text{C}$).

These observations demonstrate clearly that the transplanted cells may change their positions beneath the cuticle with respect to landmarks on the host segment, while at the same time retaining to some extent their relative positions within the transplant (arrows in Fig. 1). The running time-lapse films give the impression that the transplanted cells near the margin move considerably during the motile phase. In bigger transplants the more central regions seem to be

rotated passively. This impression is compatible with the data derived by scoring several parameters at intervals (Fig. 3a and b). It therefore seems possible to explain pattern reconstitution in *Dysdercus* without assuming some kind of resetting (in this case of pigment synthesis) in the transplanted cells. Finally it may be pointed out that the change of the angle between segment margin and the red/white border corresponds to changes in ripple orientation described by Lawrence *et al.*³ in *Rhodnius*. Should the changes in this insect also be due to cell migration in the marginal parts and rotation of the central parts of the transplant, then some inconsistencies between the observed patterns and those simulated by the computer on the basis of the resetting hypothesis (their Figs 8a and 19) might be due to migration of the marginal transplant and perhaps host cells.

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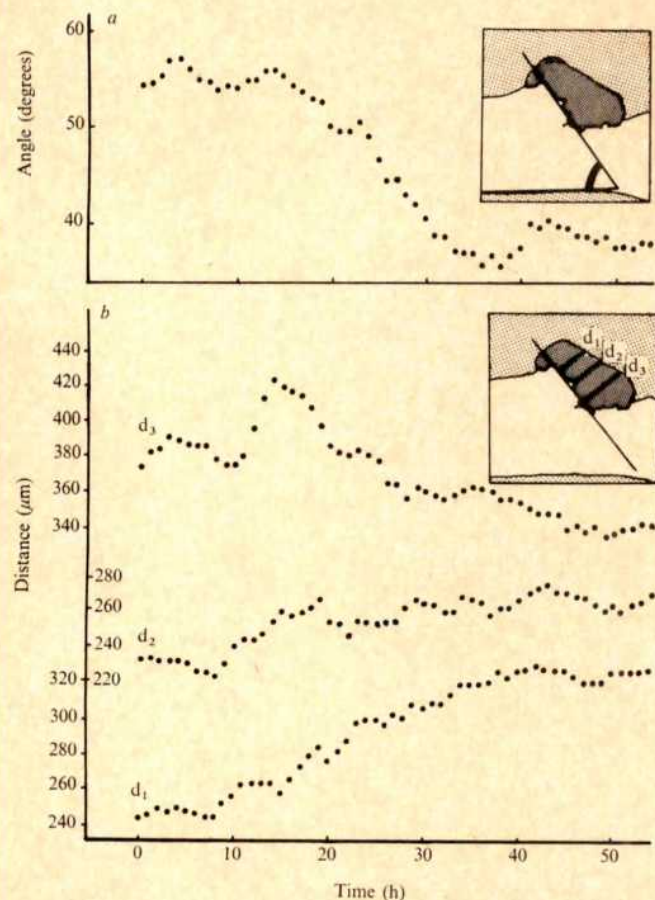


FIG. 3 Analysis of the time lapse film mentioned in Fig. 2. a, Change of the angle between the red/white border and the posterior segment margin; b, change of the dimensions of the red patch along the lines marked as d_1 , d_2 and d_3 (see inset, cf. Fig. 2a). The curves are smoothed by running averaging over three terms.

Left-handed subunit helix in flagellar microtubules

ONE of the problems encountered in the study of a small negatively stained biological object by electron microscopy concerns the uncertainty in the distribution of stain around the object. If the particle is considered broadly as having two 'sides' one in contact with the support film and the other not, then the staining conditions may produce an image which represents one or other of the two surfaces or both sides together. Generally, the degree of contrast lies somewhere between these extreme cases¹. Unless there is a differential staining of one side with respect to the other and an unambiguous distinction between the near and far sides can be made it is generally impossible to determine the right or left-handedness of an object. (Moody² has however been able to demonstrate the right-handed sense of bacteriophage T4 sheath by the use of a convenient staining artefact).

A straightforward method for determining the sense of a helical object has been described recently by Finch³ in an investigation of negatively stained TMV particles, and in the present study this technique has been applied to the microtubules from the flagella of *Chlamydomonas reinhardtii*. The method depends on the fact that as a helix is tilted about an axis perpendicular to its own axis, either towards or away from an observer, the projected image becomes asymmetric about the centre line. More precisely, the helix lines at the edge of the image on one side become more inclined with respect to the axis and less so on the other. The difference in appearance between the two sides is most marked when the tilt is equal to the pitch angle of the helix which is, for a

microtubule, approximately 12° – 15° . This is the most obvious helix in the negatively stained structure and is defined as that with the smallest pitch passing through subunits in adjacent longitudinal filaments⁴.

A short section of central microtubule from a *Chlamydomonas* flagellum is shown in Fig 1. The microtubule, negatively stained with uranyl acetate, has been tilted towards (positive) the observer by 12° in the upper micrograph and in the opposite direction (negative) in the lower micrograph. The top end of the microtubule, as it appears in the

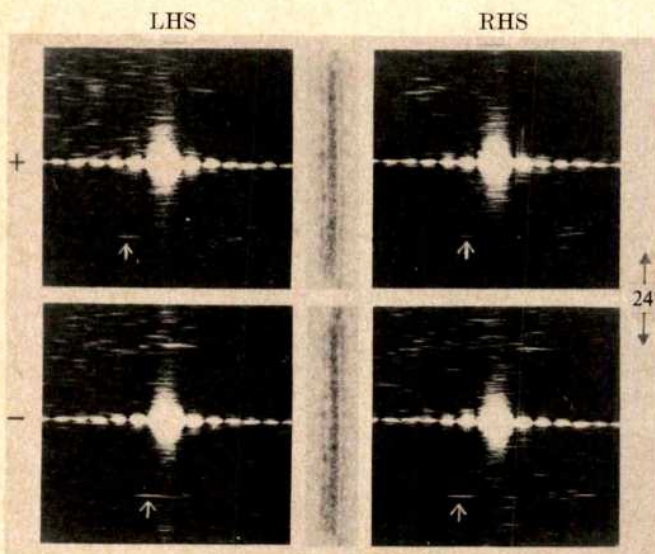


FIG. 1 A short section of negatively stained central microtubule from a *Chlamydomonas* flagellum tilted, by 12° , towards (+) and away (–) from the observer. The optical transforms are of the right (RHS) and left (LHS) hand side of each micrograph and the major 4 nm reflection is shown in each by an arrow. The reflection lies closest to the meridian for RHS+ and LHS– (particularly clear for the negative tilt) indicating a left-handed helix of subunits.

figure, moves towards the observer for positive tilt and away for negative tilt. Clearly the micrographs themselves do not reveal any distinctive change in pattern across the microtubule diameter but optical transforms of each half (RHS and LHS) of the two images are capable of resolving the differences. A strong reflection from the 4 nm axial periodicity in the microtubule^{4,5} is arrowed in each transform and the radial positions may be compared in the two transforms from each micrograph. (The presence of only one strong reflection close to the meridian in each pattern signifies predominant staining of only one side of the microtubule.) It can be seen that the reflection lies closest to the meridian for RHS positive and LHS negative and this is compatible with a left-handed helix³. The differences are small because of the flattening of the microtubule but are, nevertheless, distinct. Additional reflections further away from the meridian appear in some of the transforms and their relative positions also change with the degree of tilt. These can also, in principle, be used to determine the helix sense but as they correspond to families of subunit helices of greater pitch, the microtubule would have to be tilted through a considerable angle (40° – 50°) before any measurable change could be detected in the radial positions of the diffraction maxima. Since the image quality deteriorates rapidly with increasing tilt angle observations have to be restricted to the near-meridional reflections. Of the transforms so far examined those that contain discrete 4 nm reflections have shown consistently a left-handed helix of subunits, although no conclusive results have as yet been obtained for microtubules from sea urchin sperm flagella.

Once the helix sense has been determined it is also possible to infer from the 'sense' of the transforms which side of the microtubule is stained in a one-sided image. The diffraction patterns in Fig 1, and the majority of those obtained, indicate for this preparation predominant staining of the surface away from the specimen grid.

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Suppressor cells in normal immunisation as a basic homeostatic phenomenon

WHEN guinea pigs are treated with one dose of cyclophosphamide (CY, 300 mg kg⁻¹) 3 d before sensitisation with either ovalbumin in Freund's incomplete adjuvant (OA/FIA) or 2,4 dinitrofluorobenzene (DNFB), the resultant Jones-Mote or contact skin reactions elicited 1 week later are increased in intensity and induration and prolonged as compared to control animals^{1,2}. We have postulated that CY pretreatment depletes a population of cells which normally modulate contact sensitivity and Jones-Mote reactions^{1,2}. If CY was depleting a population of suppressor cells normally present at the time of sensitisation, the passively transferred cells from these animals should be more reactive than if there was no CY given to the donors. Passive transfer of spleen or peritoneal exudate cells (PEC) from animals sensitised with OA/FIA 8 d earlier caused more intense and more indurated reactions in normal recipients when donors had been pretreated with CY 3 d before the OA/FIA sensitisation, when compared with those reactions transferred from sensitised animals which had not received CY (S. I. K., D. P., and J. L. T., unpublished) (Fig. 1). We used the following model for the assay of suppressor cells. Guinea pigs, treated with CY 3 d before immunisation with OA/FIA, served as cell recipients 8 d later. They were then potentially susceptible to the effect of suppressor cells which they were lacking.

The recipient animals in all experiments were 450–500 g albino Hartley strain guinea pigs. They were all treated

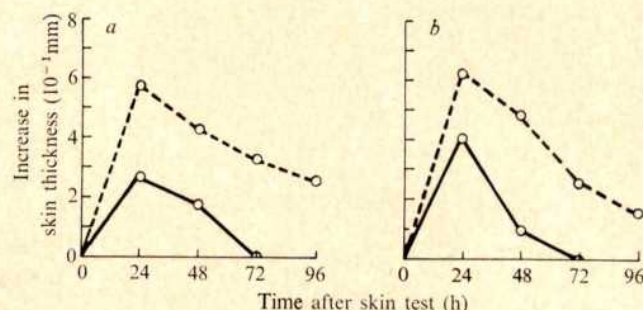


FIG. 1 Passive transfer into normal animals of cells 8 d after sensitisation with OA in FIA with (○—○) or without (○---○) CY pretreatment. There were five recipients in each group. a, spleen cells; b, PEC.

TABLE 1 Skin reactivity of animals treated with CY 3 d before sensitisation with OA in FIA, serving as cell recipients 8 d after immunisation

Donor immunised with	Days after sensitisation donor cells transferred	CY pretreatment of donor	Viable No. cells Spleen	PEC	No. of recipients	Increase in skin thickness*
No donor	—	—	—	—	11	4.6
OA/FIA	8	No	3×10^8	—	8	2.6
OA/FIA	8	No	—	2×10^8	8	1.8
OA/FIA	8	Yes	3×10^8	—	5	6.7
OA/FIA	8	Yes	—	2×10^8	5	6.0
BGG/FIA	8	No	3×10^8	—	5	5.2
BGG/FIA	8	No	—	2×10^8	5	5.2
OA/FIA	14	No	3×10^8	—	5	1.7
OA/FIA	14	No	—	2×10^8	6	1.9
OA/FCA	8	No	3×10^8	—	5	1.8
OA/FCA	8	No	—	2×10^8	5	1.5
OA/FIA	8	No	0†	—	5	5.4
OA/FIA	8	No	—	0†	4	5.0

* 24 h after skin test with 100 μ g OA (10^{-1} mm).† 3×10^8 spleen cells and 2×10^8 PEC heat killed before transfer.

with 300 mg kg^{-1} CY intraperitoneally (LD_{50-50}) 3 d before footpad injection with 1 μ g OA in FIA (evenly divided in each footpad). Eight days after sensitisation, these animals received an intravenous injection of thrice washed spleen cells (3×10^8 viable) or PEC (2×10^8 viable) from sensitised donors. Skin tests with 100 μ g OA were performed 1 h after cell transfer and the skin test reactivity was assessed as described previously³. One group of CY treated OA/FIA sensitised animals always served as controls and did not receive any cells.

When recipient animals received either spleen cells or PEC from donors sensitised with 1 μ g OA in FIA 8 d before, the skin test reactions were markedly reduced as compared to control animals not receiving cells and to those recipients receiving cells from animals sensitised with 1 μ g bovine gamma globulin (BGG) in FIA 8 d before (Fig. 2). When the donor animals were treated with 300 mg kg^{-1} CY 3 d before immunisation with OA/FIA and the cells taken for transfer 8 d later, the skin tests of the recipient animals were increased as compared with the control animals not receiving cells and with the animals receiving cells from OA/FIA sensitised animals which had not received CY (Fig. 3). This is explicable on the assumption that, as a result of CY pretreatment of the donors, the transferred cells lacked a population of suppressor cells. The increase in reactivity would therefore result from the transfusion of effector cells alone. The results of all assays for suppressor cells using this system are shown in Table 1. Cells taken from animals 14 d after immunisation with OA in FIA and transferred into CY pretreated OA/FIA animals also decreased the reactivity of the recipient animals. Cells taken from animals immunised with 10 μ g OA in Freund's complete adjuvant (FCA) suppressed the reactivity of the recipient animals. When cells from donors immunised 8 d earlier with OA/FIA were killed by heating at 56° C for 40 min (spleen < 1% viable, PEC < 2% viable as assessed by Trypan blue exclusion) they did not suppress the reactivity of the recipient animals. Other control experiments demonstrated that cells taken from OA/FIA animals had the same reactivity in recipients whether or not the recipients were treated with CY 11 d before cell transfer.

These findings demonstrate that during normal sensitisation with OA in FIA or FCA, suppressor as well as effector cells are stimulated. The suppression of skin test reactivity in this system depends on live cells and is immunologically specific. Cells from animals sensitised to OA in FIA 7 or 8 d previously can transfer Jones-Moté type reactions to normal animals. The reactions observed start 6–8 h after skin test, reach a peak at 24 h and are markedly reduced or gone at 48 h.

It is difficult to demonstrate delayed hypersensitivity (Jones-Moté) reactions in animals sensitised with OA/FIA 14 d previously as, owing to the presence of humoral antibody, they show strong Arthus reactivity. Lymphocytes from these animals cannot transfer delayed type reactivity. If suppressor cells and antibody production are not allowed to develop as a result of CY pretreatment, however, guinea pigs show delayed hypersensitivity which can be transferred with lymphocytes to normal recipients (S. I. K., D. P., and J. L. T., unpublished). Thus, using the optimal immunisation schedule for the generation of Jones-Moté reactions⁴, effector as well as suppressor cells are normally demonstrable 7 or 8 d after sensitisation, whereas the balance of suppressor to effector cells 14 d after immunisation may be such that no delayed hypersensitivity reaction is demonstrable. This effector-suppressor balance may explain the evanescence of the Jones-Moté type of delayed hypersensitivity reactions. The demonstration of cells able to suppress delayed hypersensitivity reactions 14 d after sensitisation with OA in FIA may also explain the findings of Loewi, Holborow and Temple⁵ who showed that split tolerance or immune deviation to OA (no delayed hyper-

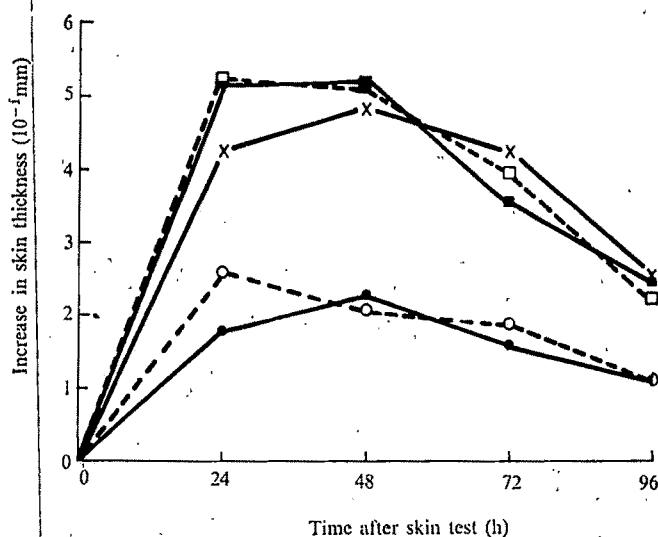


Fig. 2 Transfer of PEC and spleen cells. Donor cells taken 8 d after sensitisation with OA or BGG in FIA. Recipient animals, which were treated with CY 3 before immunisation with OA in FIA, received cells 8 d after immunisation: ●—●, OA/FIA PEC (8); ○---○, OA/FIA spleens (8); ×—×, controls (8); ■—■, BGG/FIA PEC (5); □---□, BGG/FIA spleens (5); () number of recipients.

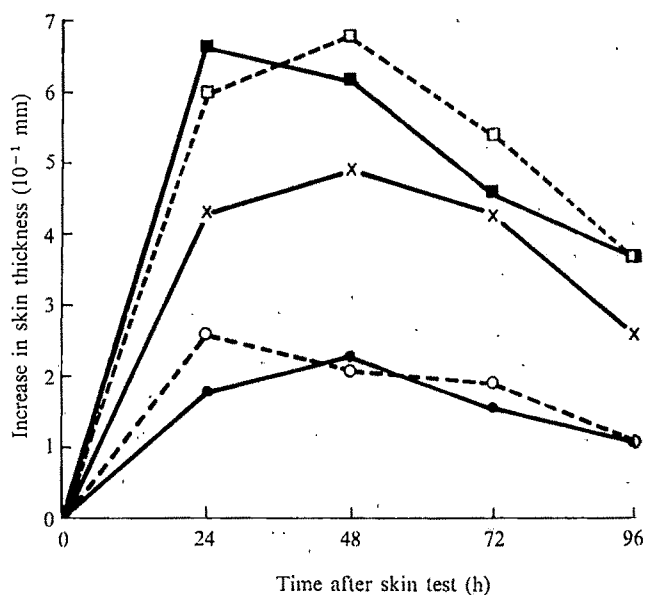


FIG. 3 Transfer of PEC and spleen cells. Donor cells taken 8 d after sensitisation with OA in FIA with or without CY pretreatment. Recipient animals, which were treated with CY 3 d before immunisation with OA in FIA, received cells 8 d after immunisation. •—•, OA/FIA PEC (8); ○—○, OA/FIA spleens (8); ×—×, controls (8); ■—■, CY—OA/FIA PEC (5); □—□, Cy—OA/FIA spleens (5). () number of recipients.

sensitivity reaction while antibody production is normal) follows 14 d after immunisation with OA in FIA. Although we had not anticipated the ability of cells from animals immunised with OA in FCA to suppress delayed hypersensitivity reactions, their presence is in keeping with our earlier studies². It may be that many more effector cells are stimulated by OA in FCA as compared to OA in FIA and when suppressor cells are depleted by CY pretreatment of OA/FCA animals the effect of this depletion is much more difficult to demonstrate.

What are these suppressor cells? Our earlier studies on the morphological and functional effects of one dose of CY show depletion of B cell areas from lymphoid tissues of mice and guinea pigs and a decrease in antibody formation^{1,2,6}. There is also a selective increase in θ -positive cells in CY-treated animals⁷. All this would indicate that CY has a selective effect on B lymphocytes and would favour the postulate that the suppressor cells are B cells as they are depleted with one dose of CY before sensitisation. CY may also affect short lived T lymphocytes however, and for this reason we thymectomised adult guinea pigs, sensitised them 6 weeks to 3 months later and found no change in skin test reactivity to OA or DNFB 7 d after sensitisation with OA/FIA or DNFB. By extrapolation from findings in the mouse, adult thymectomy should deplete short lived or T₁ cells⁸ and, if these were the suppressor cells, we should see changes in the skin test reactivity. The assumption that this procedure (adult thymectomy) should have the same effect in the guinea pig as in the mouse may be wrong.

The evidence thus favours the existence of B lymphocyte suppressor cells being stimulated during normal immunisation. These suppressor cells are acting at the efferent end of the delayed hypersensitivity reaction as skin tests are performed just 1 h after intravenous injection of cells. Several possibilities exist as to how this suppression may occur. First, in all the normal immunisation systems in which suppressor cells have been demonstrated there is eventual antibody production and it is possible that small amounts of antibody coat cells which then come into contact with the antigen and compete with effector cells for antigen. Moreover, it has not been possible so far to dissociate suppressor cell

from humoral antibody formation. Second, the suppressor cells may liberate substances which inhibit the effector cells or their products. Third, suppressor cells may liberate substances which are chemotactic for other cell types which might modulate the normal reaction. In this regard we have recently confirmed the work of Dvorak *et al.*⁹ who have demonstrated that basophils are found in high percentages in all of these skin test reactions when elicited early after immunisation.

Effective cell separation techniques for guinea pig cells will help determine, with greater certainty, which lymphocyte subpopulation is responsible for the suppression of delayed hypersensitivity reactions during normal immunisation. The mechanisms⁴ by which this suppression occurs could then be more easily determined.

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Pyrrolizidine ester alkaloid in danaid butterflies

THE dihydropyrrolizines (I-III, Fig. 1a) found in the hair-pencil secretions of male butterflies of the sub-family Danainae¹⁻⁵ and shown in one instance to be an important courtship pheromone⁶, are closely related to the hepatotoxic dihydropyrrolizine metabolites of the pyrrolizidine alkaloids^{7,8}. We have previously adduced evidence which points strongly to these hair-pencil constituents having their origin in plant alkaloids (or their metabolites) ingested by the adult insects⁵. Thus, there are a number of reports of adult male danaid butterflies being strongly attracted to, and sometimes feeding on, dead and withering plants containing pyrrolizidine alkaloids⁵. With one species, *Danaus chrysippus petilea* (Stoll) we have shown⁵ that when dihydropyrrolizine-deficient laboratory-reared butterflies are given access to one such alkaloid containing plant, *Heliotropium amplexicaule* (Vahl), they soon acquire the dihydropyrrolizine ketone (I) which is normally found on the hairpencils of this species.

We report here the first identification of a pyrrolizidine ester alkaloid, lycopsamine, in hair-pencil extracts of two Australian danaid species captured in the field. This alkaloid is typical of those occurring in the plants to which male danoids show specific attraction. The additional molecular

complexity of the alkaloid compared with the dihydropyrrolizine phenomones and the fact that the type of branched chain acid esterifying the pyrrolizidine aminoalcohol is unique in nature to these plant alkaloids, leaves no doubt that the alkaloid is of plant origin. Its occurrence in hairpencil extracts is regarded as confirming that the dihydropyrrolizine phenomones are metabolites of plant alkaloids of the pyrrolizidine group.

Five male *Danaus hamatus hamatus* (Macleay) and twelve male *Euploea tulliolus tulliolus* (Fabricius) were captured at Wallaville, Queensland. The hairpencils of individual butterflies were removed and extracted with 30 μ l of methanol. The extracts were then individually examined, before and after conversion to trimethylsilyl derivatives, using an integrated gas chromatograph-mass spectrometer. The extracts of ten *Euploea tulliolus tulliolus* and all five *Danaus hamatus hamatus* contained the dihydropyrrolizines previously identified in these two species³ as well as a number of other components (Fig. 2). One component, common to the extracts of both species (Fig. 2a, peak 2 and Fig. 2b, peak 3) gave a mass spectrum corresponding to a viridifloric or trachelanthic acid ester of retronecine or heliotridine esterified at the C₆ hydroxyl.

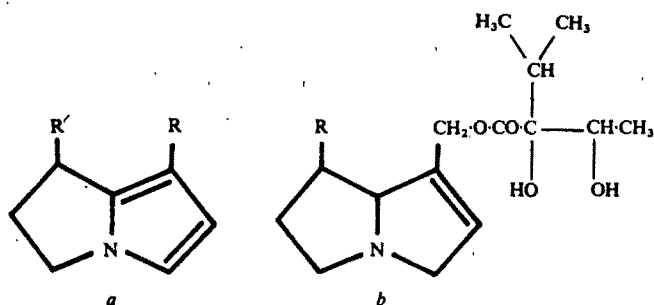


FIG. 1 a, The dihydropyrrolizines. I, R = $-\text{CH}_3$, R' = $-\text{O}$; II, R = $-\text{CHO}$, R' = $-\text{OH}$; III, R = $-\text{CHO}$, R' = $-\text{H}$. b, IV, R = β -OH ((-)-trachelanthic acid); V, R = β -OH ((+)-trachelanthic acid); VI, R = α -OH ((+)-trachelanthic acid); VII, R = β -OH ((+)-viridifloric acid); VIII, R = α -OH ((+)-viridifloric acid). (For explanation see text.)

Cochromatography of hairpencil extracts with authentic samples of the five known pyrrolizidine alkaloids (Fig. 1b) in this category⁹, that is indicine (retronecine, (-)-trachelanthic acid) (IV); intermedine (retronecine, (+)-trachelanthic acid) (V); rinderine (heliotridine, (+)-trachelanthic acid) (VI); lycopsamine (retronecine, (+)-viridifloric acid) (VII); and echinatine (heliotridine, (+)-viridifloric acid) (VIII), indicated that the butterfly alkaloid was lycopsamine (VII). The chromatographic retention time and the mass spectrum of the trimethylsilylated butterfly alkaloid also corresponded with those of authentic bis-trimethylsilyl lycopsamine.

Lycopsamine has been isolated from *Amsinckia hispida* (Ruiz et Pav.) I. M. Johnston, A. *intermedia* Fisch. et C. Mey., A. *lycopsoides* Lehm¹⁰ and *Echium lycopsis* L. (J. L. Frahn and J. A. Mills, personal communication). These plants are widely established in south-eastern Australia but occur either rarely (*Amsinckia*) or sparsely (*Echium*) in Queensland, mostly in the Darling Downs region. The known occurrences nearest to Wallaville are 180 miles distant for *Amsinckia* and 50 miles distant for *E. lycopsis* (S. L. Everist and V. K. Moriarty, personal communication). Other species which probably contain or are known to contain, this type of alkaloid occur more commonly in the Wallaville district. These include *Heliotropium amplexicaule* which is already known to attract adult male danaiids⁵ and to contain

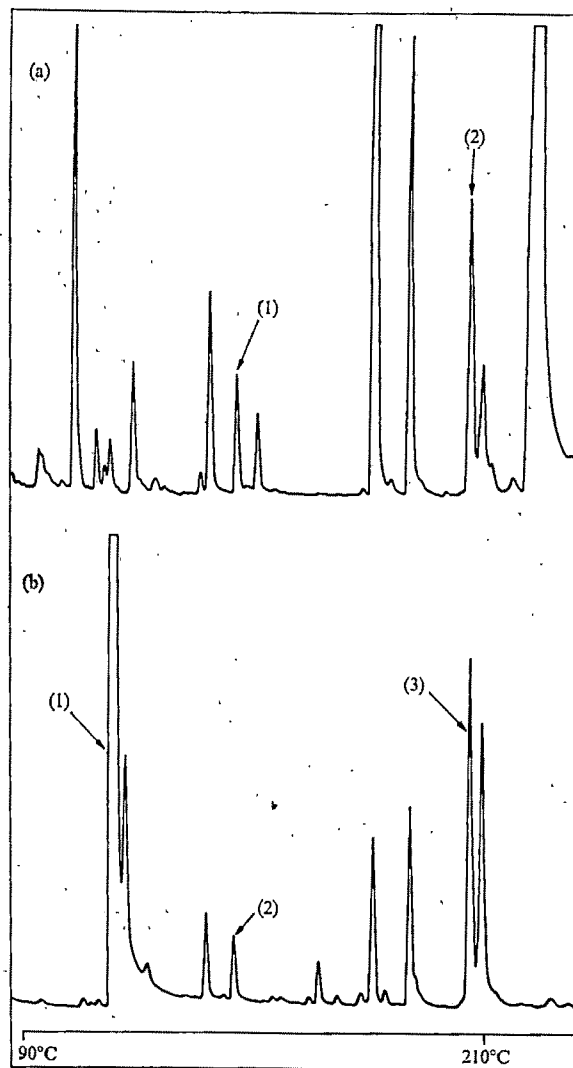


FIG. 2 a, Gas chromatogram typical of those obtained from hairpencil extracts of *E. tulliolus tulliolus* after trimethylsilylation using a 5 foot \times 1/8 inch glass column packed with 2% SE30 on Chromosorb G, mesh size 80-100. Temperature programmed from 70°C to 210°C at 4°C min⁻¹. (1), The monotrimethylsilyl derivative of 1-formyl-7-hydroxy-6, 7-dihydro-5H-pyrrolizine (II). (2) The bis-trimethylsilyl derivative of lycopsamine (VII). b, Typical gas chromatogram obtained from hairpencil extracts of *D. hamatus hamatus* after trimethylsilylation using the column and conditions described in (a). (1) 1-methyl-7-oxo-6, 7-dihydro-5H-pyrrolizine (I); (2), the monotrimethylsilyl derivative of 1-formyl-7-hydroxy-6, 7-dihydro-5H-pyrrolizine (II); (3), the bis-trimethylsilyl derivative of lycopsamine (VII). (see Fig. 1).

indicine⁹ (IV), a diastereoisomer of lycopsamine. These plants are now under investigation, as possible sources of the hairpencil alkaloid.

The requirement by male butterflies of the subfamily Danainae for pyrrolizidine alkaloids is an extraordinary fact which we believe is a pointer to their evolutionary development.

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Population estimates from recapture studies in which no recaptures have been made

THE number of animals in a population can be estimated from the proportion of individuals in a sample which have been captured previously¹⁻³. Animals which have been captured previously may be recognised by some method of marking. If the population is very large relative to the size of the samples, no marked individuals might be recaptured, because the probability of capturing any of the few marked animals is very small. Marked animals may, however, fail to be recaptured even if the population is quite small, because of random sampling error. It has not been realised before that a useful estimate of population size can be made even when no animals have been recaptured.

Suppose that a animals from a total population of size N have been marked and released. On a subsequent occasion, n animals are captured, none of which bear marks. If it is assumed that the usual conditions of recapture analysis are met, then the probability that the first animal to be captured will be unmarked is $(N - a)/N$. Similarly, the probability that the second animal to be captured is unmarked is $(N - a - 1)/(N - 1)$. Thus, the probability that all n captures are unmarked is:

$$\begin{aligned}
 P &= \frac{(N - a)}{N} \cdot \frac{(N - a - 1)}{(N - 1)} \cdots \frac{N - a - (n - 1)}{N - (n - 1)} \\
 &= \frac{(N - a)! / (N - a - n)!}{N! / (N - n)!} \\
 &= \frac{(N - a)! (N - n)!}{N! (N - a - n)!}
 \end{aligned}$$

By inserting trial values of N into this equation, one can obtain the probability P that the population is not larger than this trial value. The distribution of the probability is shown in Fig. 1, for an example in which eight marked animals have been released and several animals have subsequently been captured, none of which bear marks. As expected, the curves become asymptotic with $N = \infty$ at $P = 1$ and with $N < (n + a)$ at $P = 0$. I would suggest that in studies of small populations, an appropriate minimum estimate of population size may be made by solving the equation given above for N , by iteration, with $P = 0.5$. The meaning of this estimate is that the probability that the population

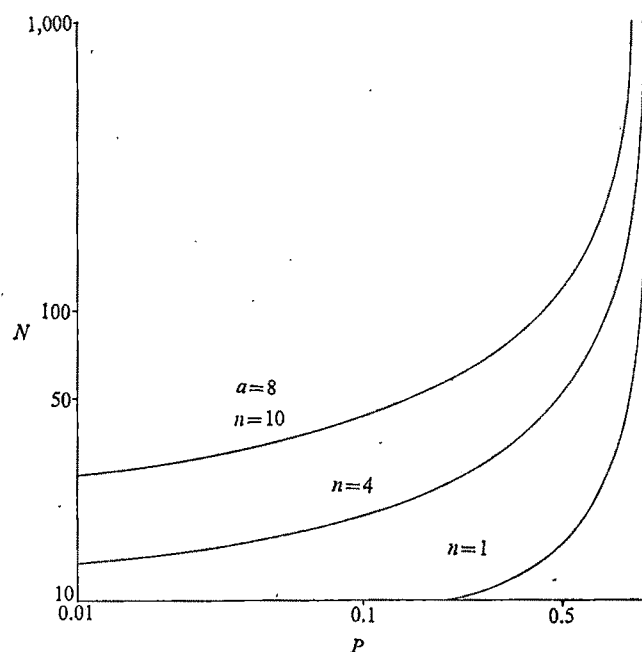


Fig. 1 Probability distribution for an example described in the text.

is larger than the estimate is equal to the probability that it is smaller. If 95% limits are required, they can be obtained by solving for N with $P = 0.025$ and $P = 0.975$ respectively.

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Restoration of libido in castrated red deer stag (*Cervus elaphus*) with oestradiol-17 β

THE role of oestrogens in male reproduction remains an enigma. It is known that the stallion^{1,2} and the boar³ excrete enormous amounts of oestrogen in their urine, and that this is of testicular origin^{4,5}; more modest amounts of oestrogen are secreted by the testis of the bull⁶, rat⁷, and man⁸⁻¹⁰. Interest in male oestrogen was recently reawakened by the discovery that the hypothalami of rats, rabbits, monkeys and men are capable of aromatising testosterone to oestradiol-17 β (ref. 11). This indicated that oestradiol-17 β might be the central mediator of androgenic effects, an idea that is beginning to receive support from a number of different experiments. For example, it has been established that newborn female rats can be sterilised equally effectively by small doses of either androgen or oestrogen¹². In addition, the anti-oestrogen MER-25 will effectively inhibit this neonatal sterilisation even when it has been induced by testosterone¹³. It also seems significant that androgens which cannot be metab-

olised to oestrogen, such as dihydrotestosterone (DHT), have no apparent effect on the brain¹²⁻¹⁶, although they are potent stimulators of male secondary sexual characteristics. Whilst DHT alone cannot restore libido to a castrated male rat, the addition of a few microgrammes of oestradiol will restore libido completely^{17,18} and, recently, it has been shown that pharmacological doses of oestradiol benzoate are sufficient to restore all components of male reproductive behaviour to castrate male rats²⁰.

We decided to investigate the effects of oestradiol-17 β on the social and sexual behaviour of free-living, wild red deer stags on the island of Rhum, off the west coast of Scotland. Although red deer may seem rather unusual experimental animals, they are particularly suitable for this type of investigation for a number of reasons. Changes in social hierarchy can easily be quantitated within the bachelor group in which stags associate for most of the year^{21,22}. Sexual behaviour is virtually confined to the rut in September and October, in which the final act of copulation is preceded by several weeks of elaborate and well-defined behavioural displays. These include roaring, flehmen, wallowing in peat hags, thrashing the herbage with the antlers, repeated 'flicking' of the penile sheath, protrusion of the penis with emission of spurts of urine which soak the ventral body wall and impart the characteristic strong rutting odour, migration from the home range to a traditional rutting area, herding of groups of hinds, and intense aggressive displays frequently leading to fights with other stags^{21,22}. Testosterone appears to exert a dose-dependent inductive effect on the level of social aggression, whereas it has a permissive effect on sexual behaviour, which can only be exhibited at certain times of the year²². Castration of a stag even only a few weeks before the rut will eliminate every component of sexual behaviour, which can be fully restored with an implant of testosterone²². Finally, the antlers of the stag serve as a convenient external indicator of its endocrine state, as antler casting in the spring is a result of a testosterone deficiency, and can be produced experimentally by castration, whereas cleaning of the velvet from the growing antler in late summer is a consequence of rising testosterone levels²².

We carried out experiments on two intact and three castrated adult stags, each of which was given a subcutaneous implant of 100 mg oestradiol-17 β (Organon) at various times of the year; castrate and intact animals were implanted in early January and late August, and another castrated animal was implanted in early July.

The intact stags started to roar about 3 weeks after receiving the implants, and they kept this up for many months, whereas untreated normal stags never roar outside the rut. There was no initial change in the social rank of the implanted animals, and at no time did they show any signs of oestrous behaviour²³, or attract the sexual attention of other stags. They rutted normally at the usual time, but they failed to cast their antlers for at least 2 yr, giving some indication of the longevity of the implants. They gained a temporary advantage over normal stags when the latter were

casting their old antlers and growing new ones in velvet, and so they rose temporarily in the hierarchy.

The castrated animals responded to the implants by cleaning the velvet from their antlers 3 to 4 weeks later, and starting to roar. They then went on to show most of the components of rutting behaviour, including flehmen, wallowing, thrashing, flicking the penile sheath, and urination from the protruded penis. They also began to herd hinds in a manner characteristic of a rutting stag, and displayed and fought with other stags. They failed to develop a male rutting odour, however, and failed to migrate out of their home range, nor did they sustain their rutting activity for more than a few hours at a time.

Because of the practical difficulties in observing the mating behaviour of the castrates in the wild, one castrate was implanted with oestrogen whilst confined to a 15 hectare fenced enclosure on Rhum. This animal had spent a number of years in the enclosure whilst intact but vasectomised, when he had shown full normal libido. Following castration in August 1972, all components of male sexual behaviour immediately disappeared, and he cast his antlers and even became subordinate to a group of hinds in the same enclosure. He was implanted with 100 mg oestradiol-17 β on July 8, 1973, and cleaned his antlers during the first week of August. He then rapidly reasserted his dominance over all the hinds, but remained subordinate to two intact stags. When these stags were removed from his enclosure, the castrate immediately took possession of the hinds, showing the complete repertoire of rutting behaviour including mounting of hinds in oestrus, intromission, and ejaculation. At the height of the rut in October the castrate engaged in a vicious and prolonged fight with one of the intact stags that had been allowed temporary access to his enclosure.

To establish whether this resurgence of sexual activity was a direct effect of the oestrogen implant, or a result of androgen secretion by the adrenal glands or a testicular rest, the castrate was killed on October 22 and subjected to a detailed post-mortem examination; the results were compared with those from normal stags and an untreated castrate killed at the same time of year²⁴ (Table 1). Peripheral blood samples were collected and oestradiol-17 β and testosterone measured by radioimmunoassay. The weight of the oestradiol implant at autopsy was 89.4 mg, indicating that a total dose of 10.6 mg had been absorbed over a period of 106 d, giving an average daily release of 100 μ g. The concentration of oestradiol in the peripheral blood of this animal was 38 pg ml⁻¹, whereas it was undetectable (< 5 pg ml⁻¹) in the peripheral blood of an intact stag during the rut.

No testosterone could be detected in the peripheral blood of the castrate (< 0.1 ng ml⁻¹), in striking contrast to the levels in intact rutting animals (8.9 ng ml⁻¹), so it seems safe to conclude that the rutting behaviour of the castrate was indeed produced by oestradiol-17 β (or some metabolite) acting directly on the brain. The oestrogen had also apparently caused some stimulation of the seminal vesicles and limited fructose synthesis, but it had completely failed

TABLE 1 Comparison of hormone levels and secondary sexual characteristics of intact stags, a castrate stag, and an oestrogen implanted castrate stag in October.

	Combined weight of seminal vesicles (g)	Fructose content of seminal vesicles (mg/100 g)	Peripheral plasma oestradiol-17 β (pg ml ⁻¹)	Peripheral plasma testosterone (ng ml ⁻¹)	Neck girth (cm)	Mane length (cm)	State of antlers	Presence of rutting odour
Intact stags	68* (4)	285* (4)	< 5	8.9* (5)	64* (6)	10* (6)	Hard horn	+++
Castrate stags ²⁴	8	30			50	4	Velvet	—
Oestrogen implanted castrate stag	26	82	38	< 0.1	55	6	Hard horn	—

* indicates that the measurement is a mean, figures in parentheses indicate sample size²⁴.

to stimulate the development of the rutting odour, and the neck mane and neck musculature were only slightly developed.

In conclusion, this study has demonstrated that in the red deer stag, microgramme quantities of oestradiol-17 β are as effective as milligramme quantities of testosterone in restoring full sexual behaviour to castrated animals, and in producing antler changes. But unlike testosterone, oestrogen does not seem to have a direct effect on the induction of social aggression. Neither hormone is capable of producing sexual activity out of season in intact stags.

It remains to be discovered for the generality of mammals whether testicular oestrogens or oestrogens formed from testosterone in the central nervous system are essential for eliciting components of male sexual behaviour.

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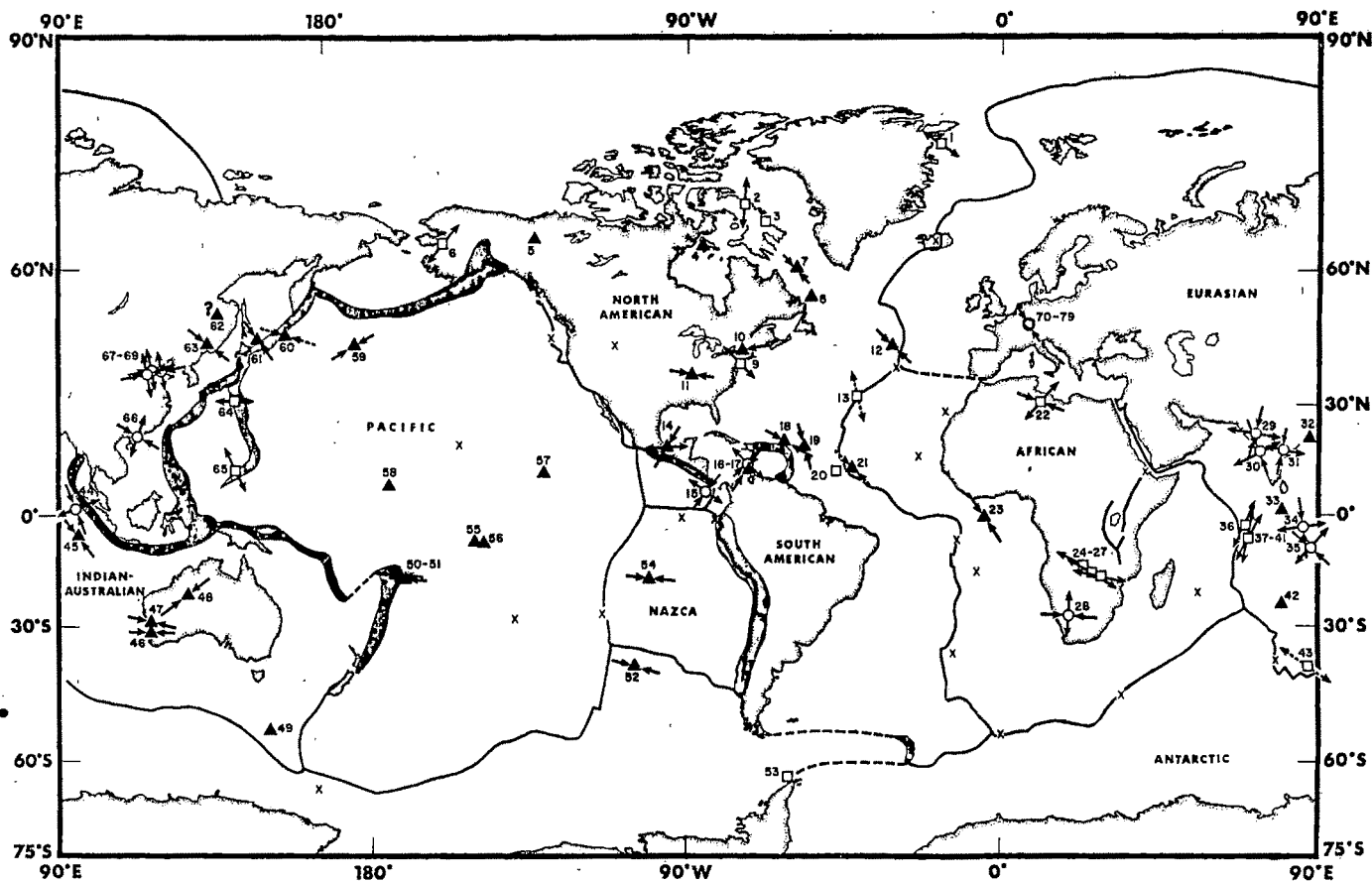
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Erratum

IN the article "Intraplate Earthquakes, Lithospheric Stresses and the Driving Mechanism of Plate Tectonics" by Lynn R. Sykes and Marc L. Sbar (*Nature*, **245**, 298; 1973), Fig. 3 was reproduced on too small a scale for the salient points to be clear and so it is reproduced again here.



book reviews

Essays on Needham and China

Chinese Science: Explorations of an Ancient Tradition. Edited by Shigeru Nakayama and Nathan Sivin. Pp. xxxvi+334. (MIT East Asian Science Series, Vol. 2.) (MIT: Cambridge, Mass and London, 1973.) £5.65.

THE purpose of this compilation of essays, according to the preface prepared by the second editor (Sivin) is twofold: to provide perspectives on the work of Joseph Needham, to whom the volume is dedicated, and to provide a sample of representative 'explorations' of Chinese science. In offering to relieve the reviewer of the burden of reading each essay, the editor invites evaluation of his book in terms of the purposes he sets out.

Because the history of Chinese science and technology has been dominated in the West by the work of Joseph Needham, the efforts to put it in perspective have been few, indeed. The measure of *Science and Civilisation in China* is best taken in terms of its purpose, which Needham himself describes in his essay in the present volume:

"One of the greatest needs of the world in our time is the growth and wide dissemination of a true historical perspective, for without it whole peoples can make the gravest misjudgments about each other. Since science and its applications dominate so much our present world, since men of every race and culture take so great a pride in man's understanding of Nature and control over her, it matters vitally to know how this modern science came into being . . . distinctively modern science did in fact come into being only in Western Europe during the 'scientific revolution' of the fifteenth and sixteenth centuries, culminating in the seventeenth. But this is indeed far from being the whole story, and to tell this part of it alone is to be deeply unjust to the other civilizations."

Needham has begun a preliminary exploration, according to the present editor, putting forward tentative hypotheses, rather than providing a definitive summary of the Chinese scientific and technical traditions. The first three essays evaluate Needham's work from different perspectives. To Derek de Solla Price, it is a "definitive classic", a systematic presentation of the raw material for generations of scholars and a point of departure for all sub-

sequent work. Shigeru Nakayama analyses the elements in Needham's thought in relation to his personal life and his views as a biochemist, which have influenced his interpretation of the history of Chinese scientific and technical development: an evolutionary rather than a mechanistic viewpoint; a preference for synthesis over analysis; and dialectical materialism. These, in Nakayama's view, have led to "a number of insecurely based speculations", which contradict both conventional scholarship and historical facts. Summarising the views of Needham's critics and analysing the more controversial of Needham's views, Nakayama evaluates *Science and Civilisation in China* as a body of "heuristic suggestions and unelaborated ideas", which remain to be synthesised by future scholars. It is in this sense that Needham's work is a "determinant" of modern awareness of China. A. C. Graham comments on the development of Needham's response to the problem of why there was a Scientific Revolution in Europe but not in China, from a "fairly straightforward Marxist answer influenced by the early Wittfogel" [in *On Science and Social Change* (1944)] to "his sociological explanation but in a much more developed and refined form, for which he acknowledges a debt to Jean Chesneaux and Andre Haudricourt". Needham's thought on Chinese scientific development no more springs from a single origin than the modern science whose roots he traces to a number of cultures and societies.

The remaining six essays, by Mitukuni Yosida, Kiyosi Yabuuti, A. C. Graham and Nathan Sivin, Ho Peng Yoke, Beda Lim and Francis Morsingh, William C. Cooper and Nathan Sivin, and Saburo Miyasita, represent current scholarship based, like Needham's, on the use of primary documents, of "high intellectual and critical standards", in the editors' view. The body of scholarship which can meet such criteria is admittedly small, though its composition is ecumenical. An important contribution of this volume is in presenting representative works by Japanese scholars, which until now have rarely been accessible to the non-specialist in English translation. It is, as the editors acknowledge, regrettable that contemporary scholarship in China itself is not represented in this collection, but it is also to be regretted that the

reasons for this lack and, indeed, the present state of Chinese scholarship in this field are not discussed in the preface.

For the layman about to begin his own explorations in Chinese science, these papers provide a frame of reference for the monumental work of Joseph Needham by "de-mythologizing" the role of one man in studies of the science and technology of traditional China. With this collection of essays as a beginning, and further guided by the introductory bibliography with which this volume concludes, the layman's understanding of Chinese science should no longer have to be "determined" by Needham's work to the same extent. On the other hand, one of the motivations for the compilation of these essays was the editor's view that "everyone who is concerned about the central problems of our time has a stake in the deeper understanding of science in ancient China". Of the scholarship which deepens understanding of traditional Chinese science, it is surely that of Joseph Needham which extends understanding to those whose concern is the present.

G. C. DEAN

Fish chromosomes

Fish Chromosome Methodology. By Thomas E. Denton. Pp. vii+166. (Thomas: Springfield, Illinois, 1973.) n.p.

THE chromosomes of fishes are, characteristically, small in size and large in number. For these reasons they are difficult to handle and this book represents, in the main, a survey of cytological methods for the preparation and study of fish chromosomes.

One may well ask what justification there is for a book devoted to fish chromosomes alone. First, from a practical standpoint, there is unquestionably a big future in fish breeding for which a thorough knowledge and understanding of the chromosomes will be essential; particularly, as seems likely, where hybridisation between species may prove useful. Second, from a strictly cytological standpoint, there are many reports among fishes of Robertsonian changes associated with divergence in chromosome number between species within genera, between and within populations of the same species and even between somatic cells within individuals. It is by no means clear whether such widespread chromosome numerical variation,

in the Salmonidae for example, reflects some special structural organisation of the chromosomes facilitating breakage and rejoining in centromeric regions or, alternatively, is the consequence of special and intensive kinds of selection pressures which confer high premium upon change in linkage relations within complements.

Until the techniques for investigating fish chromosomes are improved these and other questions will remain unanswered. As the author implies the most promising development is the culture of leukocytes, along the lines which revolutionised mammalian cytology during the past two decades. So far the technique has proved only partly successful with fishes. In the meantime the book gives a comprehensive account of the many other techniques available. Also useful is the extensive list of chromosome numbers, involving 481 species. For the rest the book is a bit of a hotch-potch, dealing with subjects ranging from elementary cytology ("autosomes are composed of two strands or chromatids joined together by a centromere") to the use of the light microscope and the evolution of the fish karyotype. For those working on fish chromosomes the survey of methods, the chromosome list and accompanying references are, in themselves, good reasons for buying the book.

H. REES

Skin and feather

Avian Anatomy: Integument. By Alfred M. Lucas and Peter R. Stettenheim. Part 1 pp. iv+1-340; part 2 pp. x+341-750. (Agriculture Handbook 362: US Department of Agriculture, in cooperation with Michigan Agricultural Experiment Station, Michigan State University.) (US Government Printing Office: Washington, DC, 1972.) \$13 per 2 part set.

THE authors of this work point out that "even today there has not yet been published a complete description of any species of bird, either wild or domestic". It was to fill this gap, for the benefit of pathologists working on poultry diseases, that the basic studies began, nearly 30 years ago, of which these two volumes are an outcome. The Avian Anatomy Project, of which Professor Lucas is director, plans to issue a series of handbooks covering all organ systems. If the standard of those that are to come equals the first, this long-continued and costly undertaking will be fully justified.

There have necessarily had to be some limitations in the scope of the work. The main limitation is that, although the treatment of descriptive anatomy is full and critical, no attempt has been made at a full and critical treatment of special problems confront-

ing physiologists, pathologists and general ornithologists who will use these volumes for reference. The subject matter of the two volumes under review is also limited: treatment of skeletal muscles, ligaments, nerves, blood vessels and lymphatics is reserved for a later volume. The greater part of these two volumes is in fact devoted to pterylosis and ptilosis (the difference in the meaning of the two terms is carefully defined and discussed), feather structure (macroscopic and microscopic), feather development and growth, and other skin derivatives. The birds mainly dealt with, in addition to the domestic fowl, are turkey, Pekin duck, *Coturnix* and common pigeon; but the great horned owl is also treated in some detail and there are plenty of references to other birds.

The mass of detail that is provided is made digestible, indeed very pleasantly and easily digestible, by the clarity with which it is presented, both in the text and in the figures, and by the care that has been taken to subdivide and index it. A great deal of care has been taken to devise a consistent and clear terminology, and I hope that it will be followed by all subsequent research workers in the field. A valuable final section on methods includes a most useful introduction to techniques of anatomical illustration.

The two volumes are well produced and excellently illustrated with a total of 422 figures, some in three or four colours. By present standards the price is remarkably low, so that many of those who are most likely to need them should be able to buy them. All zoological libraries should possess a copy. As the authors point out in their introduction, "Descriptive anatomy, well done, does not become obsolete".

D. W. SNOW

Spectroscopy for rocks

Mössbauer Spectroscopy: An Introduction for Inorganic Chemists and Geochemists. By G. M. Bancroft. Pp. xii+252. (McGraw-Hill: Maidenhead, September 1973.) £6.95.

ABOUT two and a half years ago, a comprehensive review on Mössbauer spectroscopy was published. (N. N. Greenwood and T. C. Gibb, *Mössbauer Spectroscopy*, Chapman and Hall, London, 1971). Now, Professor G. M. Bancroft has published a textbook for undergraduates or postgraduates who are following courses in this subject. As the title suggests, the bias is towards applications of the technique to inorganic chemistry and geochemistry. This textbook for undergraduates and postgraduates is an effective complement to Greenwood and Gibb. At present not

many schools of chemistry provide courses in this subject but wherever Mössbauer spectroscopy is taught, the publication of a student's text will be welcomed.

The book commences with a chapter in which essential background material is presented and explained. The topics covered here include radioactivity, nuclear properties, the Doppler effect, and an account of the discovery of the Mössbauer effect. This short chapter is followed by a longer one dealing with the parameters that can be measured by Mössbauer spectroscopy. The isomer shift is introduced and explained and an account of the quadrupole interaction is given. Then magnetic hyperfine splitting is elucidated and a survey is given of the characteristic features of a useful Mössbauer isotope. This is followed by a chapter on how Mössbauer spectra may be obtained. Here one can find details of drives, sources, detectors, counting statistics, calibration, and computation of parameters using least-squares fitting programs. The use of Mössbauer spectroscopy as a fingerprint technique in inorganic chemistry is then discussed. This chapter is a set of case histories of successful applications of Mössbauer spectroscopy. Each example is carefully and critically explained.

From the earliest days of Mössbauer spectroscopy the variation in the precise energy of the absorption has been of use in understanding variations in electron density from compound to compound and the factors influencing this variation. In this book this parameter is called the centre shift and chapter 5 is devoted to a discussion of its correlation with the oxidation state of the Mössbauer element and the bonding scheme appropriate to it. Here and elsewhere in this book the author has gone to a lot of trouble to find illustrative examples involving as many different Mössbauer nuclides as possible.

Chapters 6 and 7 are the longest in the book and taken together comprise its hard core. The first of these concerns the relationship between the quadrupole splitting and the stereochemical arrangement of the groups about the absorbing atom. The second deals with the contribution made with the help of Mössbauer spectroscopy to a knowledge of the structure of rocks and minerals which contain iron. These chapters strongly reflect the research interests of the author: they are accordingly very detailed and comprehensive.

The remaining two chapters deal with the determination of site populations in silicate minerals and with the study of multi-phase assemblages such as lunar soils and rocks.

Each chapter incorporates a set of problems to which partial answers are provided. The book is well provided

with references. There are some useful appendices and an adequate index.

Research students using Mössbauer spectroscopy will find this book much more helpful than anything else available. It can be recommended without reservations to students taking courses in the subject. At today's prices, it is good value.

B. W. FITZSIMMONS

Past and present plants

Vegetation and Vegetational History of Northern Latin America. Edited by Alan Graham. (Papers presented as part of a symposium at the American Institute of Biological Sciences Meetings, Bloomington, Indiana, 1970.) Pp. xii+393. (Elsevier: Amsterdam, London and New York, 1973.) Dfl. 120; \$46.20.

THIS attractive volume does not, as its title suggests, cover the whole area between Mexico and Peru. Most of the material concerns Mexico, Panama and the Antilles, regions being actively studied by the workers whose papers are published here. That there is much more information for the whole region is shown by Graham's extremely valuable bibliography, which lists some 960 references dealing with the vegetational history of Latin America, and some review of the current situation throughout the area of the title would have been welcome.

Nevertheless, the book is a most rewarding mine of up to date information and ideas. The descriptions of modern vegetation illustrate several approaches, ranging from Porter's largely sociological survey of plant communities in Panama to the structural treatment of vegetation types in Vera Cruz by Gómez-Pompa, who furnishes a surprisingly useful dichotomous key for their identification and relates them to the prevailing ecological factors. Breedlove provides an elegant account of Chiapas, floristically the richest and most diverse of the Mexican provinces, relating the vegetation types to Beard's broader classification for tropical America, and Howard also describes the vegetation of the Antilles in structural terms. Howard further provides a statistical analysis of the distributions of taxa to illustrate the floristic groupings within the flora, particularly the different extra-Caribbean affinities of the floras of the Greater and Lesser Antilles. Similar approaches are used by Rzedowski to examine the relationships of the floras of arid regions in Mexico.

In some ways the palaeobotanical papers are more stimulating since they seriously consider the flaws in some current assumptions and techniques. Dilcher uses his incisive studies of the Middle Eocene floras of the Mississippi

delta to examine ways of overcoming the difficulties in deducing past climates and vegetation from the tolerances attributed to modern plants and points to the incorrect taxonomic affinities ascribed to many fossil floras, a topic repeatedly mentioned in the palaeobotanical papers. Barlett and Barghoorn, in describing climatic and vegetational changes in Panama during the past 12,000 years, summarise the controversy about evaluating changes in sea-level and demonstrate the greater difficulties of deducing climatic fluctuations from complex tropical vegetation as compared with the post-glacial history of mono-climax areas in North Temperate regions. Despite such problems, the power of palaeobotanical studies for tracing the history of modern floras is exemplified by Graham's demonstration that such temperate trees as *Alnus* and *Juglans*, now of disjunct occurrence through northern Latin America, gradually migrated southwards from the south-eastern United States, where they date from the Eocene, to reach northern South America by the latest Pliocene. Unfortunately, the vexed question of the means of dispersal is not discussed.

The book is well produced, excellently illustrated and, unhappily, boldly priced. It deserves to be widely read.

D. M. MOORE

Electronics made easy

Electronics in the Life Sciences. By Stephen Young. Pp. 198. (Macmillan: London and Basingstoke, August 1973.) £5.50 boards; £2.75 paper.

WHEN I visited Stephen Young in his laboratory in Edinburgh a few years ago, I was first struck by the size of his Faraday cage, and then, on entering the cage, by the variety of superbly engineered home-built equipment that he was using to record the eye movements of a small crustacean. Here was an engineer as well as a scientist, a man who took as much pleasure in designing and building equipment as he did in carrying out experiments. Now he has written a book, and a remarkably good book it is too, in which he explains, in a down-to-earth practical manner, what every good biologist should know about electronics. This approach may upset purists in the field of electronics, but it is just right for experimental biologists.

The book is thoroughly up to date with many of the circuits described incorporating integrated circuits, operational amplifiers and field effect transistors. Everything is explained from first principles with the aid of clear simple diagrams of which there are more than 250. The few criticisms that I have all concern minor errors and omissions. An earth line is omitted from Fig. 8.15; the

circuit shown in Fig. 5.52 cannot function as shown because the integrator output is the wrong sign to trigger the comparator; one is not told how to add capacitors in series or in parallel or told how to solder delicate components; a spot on an oscilloscope screen is used as a light source in such a way that it would burn a hole in the phosphor. But these are small points and detract little from the book as a whole. Indeed the book is by far the best of its type that I have read and I thoroughly recommend it to all biologists who wish to know how to design simple electronic devices or learn about the circuits on which their electronic equipment is based.

W. J. P. BARNES

Kimberlites

Lesotho Kimberlites. Edited by Peter H. Nixon. Pp. xii+350. 70 plates. (Lesotho National Development Corporation: Maseru, Lesotho, 1973.) R13; \$19.

ALTHOUGH in the past decade there have been numerous papers describing kimberlites and their xenoliths, there has not been a comprehensive volume on kimberlites in the West since the classic treatise of A. F. Williams in 1932 on the kimberlites of South Africa. In this we have lagged behind the Russians whose spate of monographs on the Siberian kimberlites have added much to knowledge of kimberlites. Now *Lesotho Kimberlites* provides a comprehensive study of a part of the South African kimberlite province on which there were previously comparatively few data.

Included within this book are the results of studies by geologists of the Overseas Development Corporation, the United Nations Development Programme, academic geologists from South Africa, Europe and the United States, and mining company geologists from South Africa. Within the book are accounts of the geology and petrology of individual diatremes and dyke swarms, and a wealth of new geochemical and mineralogical data. Although the title of the book may give the impression that this is an account of a relatively small area, the subject matter of many of the papers is of much more than purely local interest. For example, the papers on the discrete nodules and sheared peridotites from the Thaba Putsoa pipe have implications for plate tectonics and the break-up of Gondwanaland; and the papers on the Matsoku xenoliths, the petrofabrics of the peridotite suite xenoliths, and the study on the opaque oxides of the kimberlite groundmass, will all become standard references in

the kimberlite literature from now on.

Considering that twenty-eight authors have been involved in the writing of this book, the editor has maintained a creditable consistency of style. Criticisms are that there could have been more thorough cross referencing between papers, and there is repetition of data in various tables. These are minor criticisms, however, and do little to detract from the overall merits of the book. The book will appeal more to petrologists than to economic geologists since, although some kimberlites are the host rock of diamond, there are only two small sections concerning diamond. The volume is well produced and the reproduction of most plates (many in colour) is high. The book is very good value and is recommended to anyone interested in kimberlite and upper mantle geology.

J. B. DAWSON

Basis for separation

An Introduction to Separation Science. By Barry L. Karger, Lloyd R. Snyder and Csaba Horvath. Pp. xix+586. (Wiley Interscience: New York and London, November 1973.) £9.75.

SEPARATION science is a relatively new name for an important branch of analytical chemistry and though the name has probably been introduced to brighten the image of analytical chemistry there is no doubt that operations classed as separation are much more scientifically based than most of the other activities to which the word science has recently been attached. Be that as it may, the authors of this book have made a bold and largely successful attempt to treat separation processes from a fundamental point of view, at the same time recognising the great diversity of experimental techniques which can actually be used.

Chromatography is undoubtedly the most versatile, sensitive and most widespread separation technique in use today but it is arguable whether roughly half this book should have been devoted to chromatography leaving the same space to cover all other important techniques: extraction, distillation, crystallisation, membrane separation, electrophoresis, particle fractionation and mass spectrometry. The bias towards chromatography makes this book of particular value to chromatographers as it presents a unified account of this subject within a wider framework, but it may be less valuable to the chemist primarily interested in, say, zone refining or electrophoresis.

Balance aside, the really significant contribution made by the authors is their emphasis that the same basic physico-chemical principles apply to widely differing separation techniques.

These principles are outlined in the first of the three major sections of their book which covers the thermodynamic and molecular basis of distribution equilibria, the kinetics of mass transfer by diffusion and flow, and the operational aspects of the major separation methods. In the chapters of parts 2 and 3 there are repeated back references to part 1 reinforcing this emphasis on fundamentals.

Part 2 deals specifically with techniques based upon phase and distribution equilibria, and here the authors have wisely brought in outside experts to cover distillation, solvent extraction, crystallisation, ion exchange and exclusion processes, they themselves dealing with gas chromatography, liquid-liquid chromatography and liquid-solid adsorption chromatography. In part 3 they cover barrier separation processes, electrophoresis, particle separation and, very briefly, mass spectrometry.

Parts 2 and 3 present a series of reviews of uniformly high quality which contain enough theory for the basis of each technique in fundamental physical chemistry to be clear. Yet the theory does not obtrude and the book is most readable for all of its nearly 600 pages. Each chapter contains a useful bibliography listing full-length texts and the numerous original papers cited in the text.

As a chromatographer, I was particularly impressed by the authoritative and up to date treatment of the different forms of chromatography; in this respect the book is much more than an introduction and presents many new and original ideas.

This book will undoubtedly find an important place in the library of the analytical chemist and particularly in the chromatographer's, and it will provide a stimulus to the teaching of separation science. At 1.7 pence per page it is good value.

J. H. KNOX

Pyrethrum

Pyrethrum: The Natural Insecticide. Edited by John E. Casida. Pp. xvii+329. (Academic: New York and London, December 1973.) \$16; £7.70.

THIRTY years ago, a variety of insecticides of vegetable origin were in use; but virtually all of them except pyrethrum were gradually displaced as modern synthetic insecticides became available. Despite the well publicised adverse effects of these new pesticides, it is likely that they will be needed for some vital uses, for some time to come. But there is no doubt that the present climate of opinion is very favourable for pyrethrum as a non-persistent natural product; hence this book. The virtual restriction to natural pyrethrum

is perhaps unfortunate, in view of the highly promising synthetic pyrethroids recently developed in Britain.

The 17 chapters of the book examine the present knowledge of pyrethrum and its active constituents from various points of view. The first two papers, by industrial experts, trace the commercial history of pyrethrum and the present sources of production. There follow three excellent chapters on chemistry. The composition of pyrethrum extract from the flower heads and methods of analysis and assay are summarised by S.W. Head of the Pyrethrum Marketing Board of Kenya. M. Elliott and N. F. Janes then describe the stereochemistry of the active components. The existence of both geometrical and optical isomers as well as variations in structure make this a highly complex subject, relying in recent years on mass spectrometry and nuclear magnetic resonance, for confirmation of hypothetical configurations. J. Casida then deals with the biochemistry; both the synthesis of pyrethrins in the plant and their degradation pathways in animal tissues.

The next six papers deal with toxicology and pharmacology. These comprise summaries of information on, first, toxicity to mammals, then an excellent paper on the effects of synergists in regard to toxicity by I. Yamamoto, and a rather disappointing account of what is known of the actual mode of action. The three other papers in this section describe original investigations of the effects of pyrethrins on wildlife; some tests of its possible teratogenic, carcinogenic, mutagenic and allergenic action; and interactions with other drugs. In the following section, four chapters describe types of usage: for medical and veterinary pests, for agricultural, and for forestry insects. A chapter on residue and tolerance considerations (including two synergists as well as pyrethrum) is, for some reason, set in the "Summary" section. More appropriately, E. M. Mrak concludes by summarising the advantages and disadvantages of pyrethrum, leaning rather heavily towards the former.

Despite inevitable inequality of treatment, the book provides a good distillation of many complex matters as well as a useful source of references.

J. R. BUSVINE

Corrigendum

IN the book review "IQ and inequality" by Jinks and Eaves (248, 287; 1974), the sentence starting in paragraph 8, line 7 should read "Making E' applicable purely to offspring results in a significantly poorer fit to Jencks's data" instead of "...slightly poorer...".

science on television

US "Nova" less than super

Miranda Robertson

SUNDAY March 3 this year saw the first in the science series "Nova", an attempt by the WGBH-TV, Boston backed by some of the more important science grant-giving bodies, to popularise the activities of scientists. The material for the programme, which will be transmitted by most of the 234 stations of the public broadcasting service, will comprise hour-long feature films drawn from various sources and including some originating with WGBH-TV, which will produce the programmes with the cooperation of the American Association for the Advancement of Science.

A sample preview of two of the films was presented at the meeting of the American Association for the Advancement of Science in San Francisco at the end of February, just before the series was due to start. The sample comprised a BBC feature of the filming of wild life in Britain, and a WGBH-TV film on pre- and exobiology which, it cannot be denied, suffered by comparison.

To begin with, the BBC's colour was superior; and they had, in the pond and tree life of Oxfordshire, (if not in the bearded fauna of the Oxford Zoology department) the more photogenic subject. It is hard, after all, to imagine anything much less visually enthralling than a flask full of nascent amino-acids, however exciting its implications. But it was of laboratory shots of this kind, symmetrically interspersed with explanatory face-to-face monologues from such exobiological eminences as Leslie Orgel and Carl Sagan, that the film was principally composed.

The aim was to cover what can be guessed about the origin of life on Earth from the geological record and from attempts to reproduce the first chemical steps in the laboratory, as well as the current best guesses on the possibility of life elsewhere. The personnel in the field seemed fairly represented and the main ideas and experiments came over without conspicuous error or confusion.

But the film as a whole (with the possible exception of a somewhat gratuitous excerpt from Disney's "Fantasia") was visually dull, and the script

did little to compensate.

As messages in the media go, the health series planned for the Autumn by Children's Television Workshop, the makers of "Sesame Street", looks like making better television. They have, of course, the advantage of being able to recruit the talents of professional entertainers: warnings about hypertension are more arresting in the black-treacle tones of a first-rate blues singer than in the reedy ones of a medical expert.

Hypertension, in fact, has been singled out as one of the most important health hazards to US citizens, not least because it is prevalent in the black community. The other great 'national epidemic' is obesity, which, according to CTW, affects 60% of American men and 40% of American women. Hence a skit entitled "Fatman", featuring the Fatmobile and a villain called the Nibbler. The humour is unsophisticated, but then so presumably is most of the audience. More important, this public-spirited assault on some of the nation's great health problems looks like being eminently watchable.

matters arising

Small membrane-labels do not unambiguously reveal membrane 'sidedness'

SIR—Reaction of intact erythrocytes with certain small, radioactive or fluorescent organic anions labels fewer membrane proteins than similar treatment of erythrocyte ghosts. Several membrane biologists claim that this differential labelling derives solely from 'sidedness' of the membrane; they assume that ghost isolation does not change the orientation and/or intrinsic reactivity of membrane proteins.

The compounds tested however, [stilbene-4-acetamido, 4'-acetamido, 4'-thiocyano disulphonate^{1,2} (SITS); ³⁵S-diazobenzene sulphonate (DABS)^{3,4}; ³⁵S-formyl methionyl sulphone methyl phosphate (FMSMP)^{5,6} and trinitrobenzene sulphonate⁷⁻⁹], also react with the intracellular protein haemoglobin (Hb), indi-

cating that the selective labelling of membrane proteins in the case of erythrocytes cannot be simply ascribed to label exclusion. Also, dimethylaminonaphthalene-5-sulphonyl chloride (DANSCL) permeates erythrocyte membranes, combines with Hb (ref. 10), but, in intact cells, reacts only with the few membrane proteins also labelled by DABS, FMSMP and TNBS, while acetic anhydride (fully permeant reagent), labels membrane proteins equivalently in cells and ghosts¹¹; different reactivities of membrane proteins in intact erythrocytes have also been established for TNBS and picryl chloride^{8,9}.

Bretscher¹² attacks the caution^{10,11} that the differential labelling of erythrocytes and erythrocyte ghosts might reflect more than native 'sidedness'. Although the ambiguities in the use of small labels are listed¹⁰, Bretscher addresses only labelling studies with DANSCL and acetic anhydride. He ar-

gues that protons do not permeate membranes, that intracellular pH lies well below buffer pH, that the cytoplasmic membrane face thus bears only protonated NH₂-groups, which will not react with DANSCL or acetic anhydride, and that pH drops further during labelling.

Bretscher errs in his assessment of H⁺ distribution across the erythrocyte membrane and misquotes¹³⁻¹⁵. Indeed, it is established that H⁺ freely permeates erythrocyte membranes and that the transmembrane H⁺ distribution follows thermodynamic (Gibbs-Donnan) equilibrium. Intracellular pH, computed according to the Gibbs-Donnan relationship, accurately fits experimental measurements¹⁸. From this and the haemoglobin acid-base titration¹⁹, the intracellular pH for the cells in 155 mM sucrose, 52 mM Na₂HPO₄, titrated to pH 9.0 by 77 mM NaH₂PO₄ (ref. 10), approximates 8.4 and not 7.2 as implied¹². With amino

pKs near 9.5, and internal pH 8.4 this leaves many amino groups unprotonated. Dansylation proceeds even at pH 7.5 (ref. 20) well below the pH used before¹⁰. Poor dansylation at pH < 7, derives from hydrolysis DANSCL hydrolysis in aqueous solvents independent of H⁺ between pH 6.9 and 9.5 (ref. 21), and is reduced¹⁰ by use of high concentrations (2.5 mM) of DANSCL in a micellar vehicle. As H⁺ permeates erythrocyte membranes, transmembrane pH, after reaction, will still follow thermodynamic equilibrium. Even if the membrane were H⁺ impermeable, buffering by Hb (ref. 19), would keep the pH change, due to reaction of 5×10^8 sites per cell (ref. 10), to ~ 0.3 pH.

Thus, since SITS, DABS, FMSMP and DANSCL have access to and react in the cytoplasmic space of erythrocytes, these reagents may not report membrane 'sidedness' ambiguously.

Yours faithfully,
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Internal structure of Venus

SIR,—Bullen's recent reply¹ on this subject calls for further comment as it involves a number of misconceptions if not outright errors, and also some incorrect statements of fact. Only two main points will be dealt with here.

First, as a general theoretical consideration: with the mass M of Venus as datum, the only property known at present to be accounted for is the solid radius R_v as now determined by radar. If xM , yM , zM , and $(1-x-y-z)M$ are the proportions of mass in the inner core, outer core, mantle, and outermost shell, assuming four zones by analogy with the Earth, where x , y , z are all ≥ 0 , and $x+y+z < 1$ essentially, then with known pressure-density relation for each zone, the radius of the planet will in principle be some function $R(x, y, z) = R_v$. This can be regarded as the equation of a surface, in the positive octant of three-dimensional rectangular space, giving a double infinity of solutions each of which will be a possible model with correct R_v . It seems from Bullen's later full account² that the mantle and outer shell have been regarded as a single zone, so that in the present notation it would have mass $(1-x-y)M$, and instead of a surface there would be a curve $R(x, y) = R_v$ in two dimensions, giving a linear series of models with correct radius. It is not made clear how Bullen has overcome these mathematical considerations to derive a unique model. But in any event the step of regarding the mantle and outer-shell as one zone has been shown by the writer³ to affect the calculated radius of the Earth by an amount of order 100 km, and for Venus with its only slightly smaller mass, 0.815 M (Earth), the effect will not be very much less. Thus an error in radius of 50 km or more must be involved by this step, and the claim that the stated result for Venus fits the radius "extremely well" can only mean that the core values, which are highly sensitive to changes in radius, must be hugely incorrect to compensate for the oversimplification introduced for the mantle. Thus the alleged fit and identification with Fe_2O can be no more than a simulacrum, as is obvious anyway, since only the elastic constants and uncompressed densities of the materials can enter the determination of the radius.

Second, a factual point arising from Bullen's statement that "no useful favourable test of Lyttleton's theory has emerged since it was promulgated some ten years ago". This is far from being the case:

(i) My calculations went much further than the mathematical discussion of Ramsey (who did not make use of seismic data) and showed that the initial core after collapse of the planet would have less than 60% of the present radius, and that with further growth of the core to the present size would occur contraction of the surface-radius by some 300 km (ref. 3), implying a reduction of surface area by over $50 \times 10^6 \text{ km}^2$, thereby offering for the first time an amount of contraction of the order demanded by geologists for many decades to account for the numerous established periods of mountain building during the age of the Earth.

(ii) The theory predicted that Mars would be found to have no dipole magnetic field⁴ in spite of the rotation being close to that of the Earth. This was duly verified in July 1965 by the Mariner IV magnetometer, when the absence of a field was widely greeted as most surprising. (It seems that Bullen remains unaware of either the prediction or the result, which by some is considered one of the most significant achievements of the space programme, not only from his present remark but from its getting no mention in his 1969 review⁵.)

(iii) The theory also predicted that no folded and thrust mountains would be found on Mars⁶, and this too has recently become established by the Mariner IX mission.

(iv) The theory also predicts the presence of folded and thrust mountains on Venus. Although radar measures already claim large-scale features of vertical extent as much as 7 km (ref. 7), I would not regard this prediction as yet thoroughly established.

This list of successes could be seen as constituting quite favourable tests of the phase-change hypothesis, besides considerably outnumbering those achieved in upwards of a century by the iron-core hypothesis of which there seems to be exactly none other than the iterative claim that if a sample of the core could be obtained it would prove to be iron, or now Fe_2O .

Yours faithfully,
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Oxygen solubility and Henry's law

SIR,—Maharajh and Walkley reported¹ that the solubility of oxygen in water is reduced when another gas is present. The reliability of their data has been questioned on theoretical grounds² and by comparison with previously published data³. Maharajh and Walkley reported, for instance, that in a mixture of equal proportions of oxygen and krypton the oxygen solubility was depressed to less than half of its normal value. This result, if true, would be of great interest in the field of radiation biology because there are anomalous results in the literature on the effect of rare gases on the 'oxygen enhancement ratio'. We have therefore checked Maharajh and Walkley's findings, and as our results contradict theirs, we describe here our experimental arrangements in some detail.

The apparatus finally found most satisfactory is illustrated in Fig. 1. It produces a gas mixture of known composition which is bubbled through water to produce a saturated solution. A Hersch cell⁴ and associated sampling devices are used to measure the oxygen content of the saturated solution. The pressure vessel was evacuated and then filled to a known pressure with one component of the gas mixture. The second component was added to give a total pressure corresponding to the desired mixture. The mixed gas was thoroughly stirred by a fan sliding on a central rod inside the pressure vessel and was then bubbled through triply distilled water in the glass apparatus (a) shown in Fig. 1. One arm of this glass assembly couples directly into the Hersch cell through a stopcock. A sample of the water saturated with the gas mixture was withdrawn from the bubbling vessel by inserting the long needle of a Hamilton microsyringe through a flexible membrane, and then through the bore of a stopcock and into the solution. After the syringe had been flushed with the solution it was filled again and the needle was partially withdrawn and reinserted into the Hersch cell arm without the risk of any contaminating gas entering with it. A sample of 10 μ l was injected into the Hersch cell and the electric charge recorded in 3 min was measured. The Hersch cell was calibrated for oxygen sensitivity by alternating the test sam-

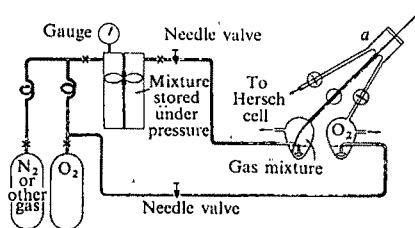


Fig. 1

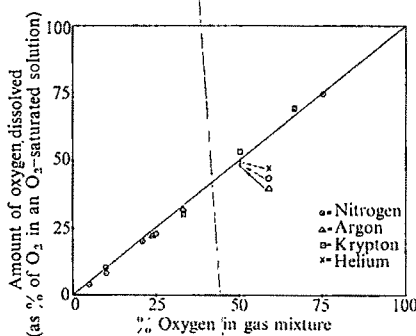


Fig. 2

ples with a series of similar samples drawn from a solution saturated with pure O₂, which was contained in the second bulb of a in Fig. 1. The experiments therefore gave a direct comparison between the amount of O₂ dissolved in the mixed solution, and that dissolved in a solution equilibrated with pure O₂.

Figure 2 shows all our results for different proportions of oxygen with several different companion gases, in relation to the straight line expected from Henry's Law. At the 50% level (equal partial pressures of oxygen and the companion gas) where Maharajh and Walkley reported 60% depression of oxygen solubility in the presence of krypton, 34% with helium and 26% with nitrogen, we found no significant departures from Henry's Law. At small partial pressures of oxygen where the accuracy of measurement in our system is lower, some points lie slightly below the line, but these deviations are probably not significant. To increase the accuracy by an order of magnitude would involve considerable expense and time, which in view of the absence of anomalies does not seem justified.

Yours faithfully,

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¹ Maharajh, D. M. and Walkley, J., *Nature*, **236**, 165 (1972).

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CS₂ and the natural sulphur cycle

SIR,—The discovery of the ubiquitous distribution of dimethyl sulphide (DMS) led to the suggestion that this compound might constitute a volatile component of the natural sulphur cycle, serving to transfer sulphur from the sea to the land¹. I report now that in addition to

DMS another sulphur compound, CS₂, is widely distributed in coastal and ocean waters.

The presence of CS₂ was revealed during routine analyses of seawater for volatile halocarbons. Analysis was by gas chromatography with an electron capture detector. An unknown peak was almost always present at a retention time suggesting a substance with a boiling point of approximately 45° C. This is the boiling point of CS₂. Other physical properties of the unknown compound were also similar to those of CS₂: the retention volume on two different columns, Henry's Law coefficient for distribution between air and water, and the rate constants for reactions with diethylamine and with gaseous electrons.

There was a search for the presence of CS₂ in the waters off the Beara Peninsula western Ireland, in July 1973. It was present in every sampled locality but the highest concentrations were in relatively stagnant bays and the lowest were where the tip of the peninsula projected into the Atlantic Ocean. None of the species of algae examined were found to be emitting CS₂ but the concentration in anaerobic mud at the sea-floor was very much higher than that in the sea above.

TABLE 1 CS₂ in seawater

Origin	Concentration (g ml ⁻¹)	No. of observations
Atlantic off Ireland	7.8×10^{-13} (4.4)*	8
Stagnant bay water	5.4×10^{-13} (3.7)	12
Mud at sea bottom	2.95×10^{-11} (1.66)	5
Open Atlantic, (50°N 65°S)	5.2×10^{-13} (1.44)	35

* Figure in parentheses are standard deviations.

Analyses of seawater during the voyage of the RRS Shackleton from the United Kingdom to Antarctica and in 1971–72 show a peak with the same retention volume as that of CS₂. Although these historical data were not subjected to the same identification procedures as the data from the Beara Peninsula, it seems probable that the unknown peak was CS₂, and the results are listed together with those of the local analyses in Table 1.

The open ocean concentrations of CS₂ and DMS are respectively 5×10^{-13} and 1.2×10^{-11} g ml⁻¹. Calculations of the rate of transfer of these compounds from the sea to the air suggest that at such concentrations neither compound is a significant source of sulphur in the atmospheric part of the natural cycle (P. S. Liss, personal communication, and ref. 2). For CS₂ this conclusion is prob-

ably correct. Dimethyl sulphide, however, shows large variations in productivity: seawater in equilibrium with the alga *Polysiphonia fastigiata* contains 10⁵ times more DMS than the open ocean. Dimethyl sulphide therefore remains a likely compound of the natural atmospheric sulphur cycle. Carbon disulphide seems to originate under anaerobic conditions on the seafloor. It is stable for at least 10 d in seawater containing oxygen, and may therefore participate in the transfer of sulphur from the seafloor back to the surface waters.

Yours faithfully,
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¹ Lovelock, J. E., Maggs, R. J., and Rasmussen, R. A., *Nature*, 237, 452 (1972).

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Watson on pterodactyls

SIR,—I wish to draw your attention to a matter concerning the obituary of D. M. S. Watson¹.

It is mentioned that Watson was a very good lecturer and that "another fine performance was his lecture on pterodactyls, which he gave on several occasions but never published".

I and my colleague (Dr George Whitfield) have been working on pterodactyls for some years. Last autumn a batch of Watson's unpublished work came to light in the archives of the University of Cambridge. We examined the notes, which included the transcript of Watson's famous lecture on pterodactyls among other material. Whitfield and I had just had our paper on the Biomechanics of *Pteranodon* accepted by *Trans. R. Soc.* and were able to prepare Watson's material (including his lecture, word for word) and asked to have it included at the end of our monograph in a separate section. The Royal Society agreed to do so. The paper will be out in the next few months. We felt this would be a tribute to him and would preserve the lecture for the many vertebrate palaeontologists who had told us they had known of Watson's work on pterodactyls, and wished he had published it.

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¹ *Nature*, 247, 496 (1974).

Announcements

Erratum

In the article "Selective binding properties of vitamin D transport protein in chick plasma *in vitro*", by R. E. Belsey, H. F. DeLuca and J. T. Potts jun. (*Nature*, 247, 208; 1974) the legends to the illustrations were transposed and that labelled Fig. 1 should be read in conjunction with the other diagram and *vice versa*.

Corrigendum

In the article "Absorption and emission by interstellar CH at 9 cm" by B. J. Robinson, F. F. Gardner, M. W. Sinclair and J. B. Whiteoak (*Nature*, 248, 31; 1974), line 2 of the legend to Fig. 1 should read "Channel spacing is 10 kHz = 0.9 km s⁻¹".

International meetings

April 29–May 3, Modern Optical Techniques and Industrial Applications (Professor J. N. Butters, Department of Mechanical Engineering, University of Technology, Loughborough, Leicestershire LE11 3TU)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Science Research Council. Publications of the Royal Observatory, Edinburgh, 1973. Vol. 9, No. 1: The 150th Anniversary of the Royal Observatory, Edinburgh, and Infrared Astronomy in the United Kingdom. Edited by M. J. Smyth and H. Seddon. Pp. 62. (Edinburgh: The Royal Observatory, 1973.) £3 net. [1712]

The Royal Society. Scientific Research in Schools Committee—Report to Council 1973. Pp. 21. (London: The Royal Society, 1973.) [1712]

Natural Environmental Research Council. Institute for Marine Environmental Research—Report 1971–1973. Pp. 74. (Plymouth, Devon: Institute for Marine Environmental Research, 13/14 St. James Terrace, Citadel Road, 1973.) [1911]

Chalk Grassland: Studies on Its Conservation and Management in South-East England. (Papers presented to a Symposium organized by the Kent Trust for Nature Conservation at Rogate Field Centre, King's College London, Rogate, Petersfield, Hampshire on 3–5 November 1972.) Edited by A. C. Jermy and P. A. Stott. Pp. 59. (Maidstone: Kent Trust for Nature Conservation, 1973. Obtainable from Major F. Abraham, Chestnut Tree Cottage, Broadoak, Mersham, Ashford, Kent.) £1.50. [1911]

Proceedings of the Royal Irish Academy. Vol. 73, Section B. No. 15: A Survey of the Plankton of Strangford Lough, Co. Down. By R. J. Boyd. Pp. 231–268. 48p. No. 16: Fossil Pingos in Camaross Townland, Co. Wexford. By G. F. Mitchell. Pp. 269–282+ plates 15 & 16. 33p. No. 17: A Study of Heat Death in Nerve-Muscle Preparations of *Rana temporaria* L. By J. N. R. Grainger. Pp. 283–290. 12p. (Dublin: Royal Irish Academy, 1973.) [1911]

The Zoological Record, 1970, Vol. 107, Section 18: Aves. Compiled by the Staff of the Zoological Society of London. Pp. vi+282. (London: The Zoological Society of London, 1973.) £9.15. [1911]

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United States Department of the Interior: Geological Survey. Professional Paper 599-H: Geology of the Sierra Madera Cryptoplosion Structure, Pecos County, Texas. By H. G. Wilshire, T. W. Offield, K. A. Howard and David Cummings. Pp. iv+42+ plates 1–4. \$1.75. Professional Paper 761: Geochemical Anomalies and Alteration in the Moenkopi Formation, Skull Creek, Moffat County, Colorado. By R. A. Cadigan. Pp. iii+21. Professional Paper 796: Structural and Stratigraphic Framework, and Spatial Distribution of Permeability of the Atlantic Coastal Plain, North Carolina to New York. By Philip M. Brown, James A. Miller and Frederick M. Swain. Pp. v+79+plates 1–59. Professional Paper 803: X-ray Mineralogy of the Parachute Creek Member, Green River Formation, in the Northern Piceance Creek Basin, Colorado. By Donald A. Brobst and Jerry D. Tucker. Pp. iv+53. (Washington, DC: Government Printing Office, 1972 and 1973.) [1311]

Meddelelser fra Danmarks Fiskeri-og Havundersøgelser. N.S. Vol. 7, pp. 75–84: On the Accumulation and Metabolization of Pentachlorophenol in Fish. By T. E. Hallas. N.S. Vol. 7, pp. 85–98: On the Prey Size of Cod and Dab. By Erik Ursin. (Copenhagen: The Danish Institute for Fishery and Marine Research, 1973.) [1401]

United States Department of the Interior: Geological Survey. Bulletin 1357: Placer Gold Deposits of Utah. By Maureen G. Johnson. Pp. iv+26+plate 1. \$1.25. Water-Supply Paper 1817-D: Preparative Free-Flow Electrophoresis as a Method of Fractionation of Natural Organic Materials. By J. A. Leenheer and R. L. Malcolm. Pp. iii+14. 35 cents. Water-Supply Paper 2026: Characteristics of Water Quality and Streamflow, Passaic River Basin Above Little Falls, New Jersey. By Peter W. Anderson and Samuel D. Faust. Pp. v+77. 70 cents. Professional Paper 562-K: An Experimental Study of Heavy-Mineral Segregation Under Alluvial-Flow Conditions. By Lawrence L. Brady and Harvey E. Jobson. Pp. iv+38. 85 cents. (Washington, DC: Government Printing Office, 1973.) [1511]

World Health Organization. WHO Food Additives Series. No. 4: Evaluation of Mercury, Lead, Cadmium and the Food Additives Amandin, Diethylpyrocarbonate, and Octyl Gallate. Pp. 84. (Geneva: WHO; London: HMSO, 1972.) 6 Sw. francs; 75p; \$1.80. [1611]

European Organization for Nuclear Research—CERN. CERN 73-13: Pion-Deuteron Elastic Scattering at Intermediate Energies. By K. Gabathuler. Pp. iv+38. (Geneva: CERN, 1973.) [312]

Royal College of Forestry, Stockholm. Studia Forestalia Suecica. No. 104: Studies on Wood Degradation and Cellulolytic Activity of Microfungi. By Thomas Nilsson. Pp. 40. No. 105: Meiotic Investigations on Embryo Sac Mother Cells of Normal and Desynaptic Norway Spruce. By Alena Jonsson. Pp. 46. No. 106: On the Ultrastructure of Needles of *Pinus sylvestris* L. By B. Waller, B. Nyman and T. Alden. Pp. 26. No. 107: Ripening Process in Relation to Temperature and Sugar Content on Seeds of Scots Pine (*Pinus sylvestris* L.). By L. Kardenli, B. Nyman and S. Bobeck. Pp. 21. (Stockholm: Royal College of Forestry, 1973. Orders to AB Allmanns Förläggare Distribution, Fack, S-162 10 Vallingby 1, Sweden.) [312]

Food Composition Table for Use in East Asia. (A Research Project Sponsored by US Department of Health, Education and Welfare: Nutrition Program, Center for Disease Control, Health Services and Mental Health Administration; and Food and Agriculture Organization of the United Nations.) Pp. xiii+334. (Bethesda, Md.: National Institute of Health; Rome: FAO, 1972.) [312]

Pacific Insects Monograph No. 30: Aethinae of Oceania (Coleoptera: Chrysomelidae). By G. Allan Samuelson. Pp. 165. (Honolulu, Hawaii: Entomology Department, Bernice P. Bishop Museum, 1973.) [312]

Fisheries Research Board of Canada. Technical Report No. 420: Marine and Brackish Water Oligochaeta. By R. O. Brinkhurst. Pp. 9. (St. Andrews, NB: Biological Station, Fisheries Research Board of Canada, 1973.) [312]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Memoir No. 366: Geology of Flat River, Glacier Lake, and Wrigley Lake Map Areas, District of Mackenzie and Yukon Territory. By H. Gabrielse, S. L. Blusson, and J. A. Roddick. Pp. 153 (45 plates). (Ottawa: Information Canada, 1973.) [312]

Office de la Recherche Scientifique et Technique Outre-Mer. Mémoires Orstom. No. 60: Le Quaternaire Cambodgien: Structure et Stratigraphie. Par Jean-Pierre Carbonnel. Pp. 248+4 planches. 100 francs. No. 65: Les Guiziga du Cameroun Septentrional: L'Organisation Traditionnelle et sa Mise en Contestation. Par Guy Pontie. Pp. 255+7 planches. 90 francs. No. 66: Les Sols de Quelques Régions Volcaniques du Cameroun: Variations Pédologiques et Minéralogiques du Milieu Equatorial au Milieu Tropical. Par Gaston Sieffermann. Pp. 183. (Paris: Office de la Recherches Scientifique et Technique Outre-Mer, 1972 et 1973.) [402]

Institut Royal Météorologique de Belgique. Publications, Serie B. No. 74: Sur Une Méthode Pratique de Décodage des Messages Météorologiques. Par J. P. Stessel. Pp. 28. (Bruxelles: Institut Royal Météorologique de Belgique, 1973.) [412]

nature

Volume 248

April 19, 1974

Lessons from Ethiopia

WE publish in this issue a report by an Oxfam-sponsored team on what can be learnt from the Ethiopian drought of last year. This is very much a first-hand report. Mr Mason and colleagues were in the area for much of the critical time last autumn when relief efforts, including their own, were turning the tide of fatalities. They are too modest about the success of the high energy food devised at Cambridge which undoubtedly brought many children back to health from the verge of death, but the greater achievement of this group and of many other devoted workers will come if their clearly spelt out recommendations are taken seriously and acted upon. It is at least highly encouraging that they report that a framework for relief can be based on established principles. An exotic or technologically complex solution is the last thing needed.

What is the prospect for 1974? Ethiopia has two harvests the first of which comes after the light spring rains. The failure of these last year led to the crisis; this year they seem to be much better in general, although the lack of seed and oxen will ensure that the harvest is small. In addition some first results from longer term aid will soon begin to prove their worth—road building and irrigation projects have made some progress. Nevertheless there are some fearfully large problems remaining. Perhaps the greatest of these is information flow.

Everybody one speaks to about Ethiopia (and this is hardly just an Ethiopian problem, although it is particularly severe there) is concerned about the lack of data. This was epitomised recently by the conflict between a Norwegian missionary's report that the south of the country was then in distress and a BBC reporter's failure to find any such situation. It is clearly inadequate that the forays of individual journalists should form the basis on which the world is alerted, and the solution is not one which poses vast technical problems, although it may well pose political ones. The sort of monitoring that nutritionists would like to see, the sort of information that would give adequate meteorological coverage, the sort of human animal and crop statistics that would give early warning of danger are such that a trained village postmaster could provide in many countries. Of course, there are no such people as yet in Ethiopia, nor are there means of communication. But it is surely not beyond the wit of a country that runs its own airline and uses computers in government to devise a suitable permanent reporting scheme. Surely in this it would have the support of international relief agencies.

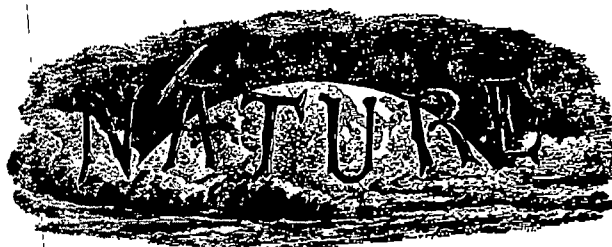
Another widely mentioned obstacle to long term progress is the problem of land use and tenure. Much of

Ethiopia's fertile land is heavily overfarmed, largely because it is parcelled up into uneconomically small units, many of which are rented out at extraordinarily high rents. Often more than 50% of the produce from this land goes in rents. The Emperor and his family, it is said, own nearly half the land in Ethiopia, and the Church a further quarter. Clearly no rational approach towards self help can ignore this source of much harm. This is one of the issues behind recent political agitations in Addis Ababa and it is difficult to see it going away spontaneously. It is to be hoped that foreign aid-giving bodies with political clout will continue to raise this matter whenever future aid is being discussed.

And what lessons are there for the aid-giving bodies themselves? It has been alarming to learn that the disaster which only broke on the world in October 1973 was known as early as April 1973 to many people who were constrained by political sensitivities to respond in a low key way. It was by no means only those in the Ethiopian government who knew, although it seems that it was they who through ignorance or touchiness underplayed the expected scale of the disaster. Nevertheless the prospect of the tragedy was known in Britain in April and even without the starkness of Mr Dimpleby's television programme, it would surely have been possible to mobilise much financial and logistic support months earlier. One has great hopes that Mrs Judith Hart, the new British Minister for Overseas Development, will look very carefully at this question. She comes to the ministry with a good reputation and a burning conviction.

She might also with profit look carefully at the ways that other countries handle aid in the field. There are tricky problems of liaison and collaboration between government and private agencies. Some have spoken well of the German approach to the operation. It is clearly a field in which all can still learn much and, one hopes, can learn quickly and without worrying about loss of face and demarcation of duties.

100 years ago



THE Chair of Chemistry in the University of Glasgow is vacant. We hope the Home Secretary in filling up the vacancy will, in the spirit which urged the late Mr. Langworthy to make the magnificent bequest above referred to, show by the appointment he makes the appreciation in which he holds original research. It is now high time that it should be distinctly understood that no man deserves to be appointed to a Chair of Science in any of our Universities unless he has shown that he has that knowledge of his subject which can only come from original investigation.

From *Nature*, 9, 490, April 23, 1874.

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The wind of hydrogen and of change blew gentle, clean and persistent at Miami...

"There was the sniff in the air of American technology on the move; much more will be heard of hydrogen before long." Professor R. W. Cahn, of the School of Applied Sciences, University of Sussex, gives his impressions of a recent conference on the 'hydrogen economy' held in Miami.

POLITICIANS and oil sheiks alike think of fuels as means of generating energy: another way of regarding them is as a means of storing energy. This approach was very much to the fore in the world's first conference on the 'hydrogen economy', organised last month by the University of Miami, with the backing of the National Science Foundation, amid the lush pleasures of the Playboy Plaza at Miami Beach. The international meeting was attended by over 700 participants, more than four times the number expected even by the buoyant organisers. Those attending were divided between the aficionados—and one of the opening speakers truly presented the adherents of the hydrogen economy as members of a quasireligious cult—and a large number of mild sceptics who were sufficiently intrigued to want to see how seriously the topic should be taken. The most popular question while sipping drinks among the bunny girls was: "And why are you here?"

Hydrogen is not a natural fuel on earth. It must first be made, most conveniently from water, and then turned back to water, generating heat (or electricity directly in a fuel cell). Since neither generation nor combustion is perfectly efficient, the cycle $H_2O \rightarrow H_2 \rightarrow H_2O$ only makes sense from three aspects: it offers a compact means of storing and transporting energy which is not conveniently used at the site and time of liberation; it provides a possibility of creating a transport-fuel with very high energy content per unit mass; and it provides one possible substitute to pass through the insatiable natural gas pipelines of North America.

Nearly 100 papers (available in a preprint volume from the School of Continuing Studies at the University of Miami) were presented broadly on the following themes: generation of hydrogen by means of nuclear, solar or geothermal energy, and specifically by electrolysis or thermochemical cycles with catalysts, or by steam reforming of carboniferous fuels; storage of hydrogen as liquid or, more especially, in thermally decomposable hydrides; transport of hydrogen through pipelines, including problems of embrittle-

Hydrogen is not a natural fuel on earth. It must first be made, most conveniently from water, generating heat, or electricity directly in a fuel cell.

ment by hydrogen; hydrogen as a fuel for cars and aircraft; the relative cost of hydrogen and other fuels, particularly methane, for various uses. Indeed, the conference was notable for the scrupulous care devoted to the economics of the various patterns of hydrogen production and use. The many attempts at costing covered a moderately wide spectrum, and one author's assumption was on occasion disproved by another author's technical innovation. Thus, one

of the numerous papers on thermochemical cycles, by R. I. Chao (University of Puerto Rico) and K. E. Cox (University of New Mexico), concluded that "thermo-chemical hydrogen via a series of closed chemical cycles and nuclear heat would cost \$1.50–2.35 per million BTU, whereas hydrogen from electrolysis/nuclear power would cost \$4.60–6.28 per million BTU" (compared with present jet fuel costs of \$1.7 per million BTU). But Chao and Cox assumed electrolysis capital costs at \$200 per kW, whereas L. J. Nuttall, A. I. Fickett and W. A. Titterton of the General Electric Company (GE), in their very impressive paper on hydrogen generation by electrolysis with solid polymer electrolyte

Hydrogen fuelled cars seemed fairly promising, but such cars would probably require a huge discontinuous change in distribution which could scarcely come by easy stages.

estimated capital costs which might be as low as \$60 per kW and overall hydrogen costs in the range of \$2–3 per million BTU (excluding liquefaction costs), which is distinctly interesting in the medium term. The GE paper was a good example of high technology transfer, since it described a thoroughly engineered process originally designed to make tonnage oxygen for the space programme, with hydrogen going to waste; this could easily be turned on its head. The GE technique effectively separates the two gases; permits extremely high current densities and, very important, gets by with exceptionally low loadings of noble metal catalyst. Ordinary commercial electrolyzers cannot afford to use catalyst and thus have to operate very inefficiently.

The numerous papers on thermochemical cycles left a overall impression of a technologically very difficult method dependent on extremely efficient recovery of catalyst and with many uncertainties as to overall efficiency of energy conversion. It is also remarkably difficult to decide an optimum series of chemical cycles for investigation: even a computer-aided search of possible cycles, by J. L. Russe and J. T. Porter of the General Atomic Company, gave conclusions which are hard to evaluate. One listener was left with the impression that electrolysis scores on points over thermochemical cycles, until the end of the century at least. This view was not weakened by a very interesting paper by J. B. O'Sullivan and others of the United States Army, who described a detailed and, hitherto unpublicised feasibility study of a mobile hydrogen generator for field use, centred on a thermochemical cycle. The full details of this are to appear in the final proceedings of the conference, to be published by Plenum Press.

Opinions were violently divided on hydrogen as a fuel for internal combustion engines. E. M. Dickson and colleagues of the Stamford Research Institute presented a closely argued critique, with pessimistic conclusions, of the use of hydrogen to fuel 'personal vehicles'. An enthusiast, R. E. Billing of the Billings Energy Research Corporation (many small companies were represented) however described in optimistic detail his own experience of operating a fleet of hydrogen fuelled motor-caravans, and he also claimed considerable

operating data of a converted passenger car working interchangeably with hydride or cryogenic storage.

Papers dealing specifically with hydrogen storage in cars were particularly interesting. Several discussed the detailed engineering design of hydride storage beds, mainly with reference to $\text{FeTiH}_{1.6}$ and MgH_2 . The first of these is heavy but decomposes at low temperatures; the second is light but needs to be heated to over 600°C . Billings explained how an optimum (i.e. lightest feasible) combination of the two hydrides could be used, with as much MgH_2 as could be sufficiently heated by the exhaust gases of the engine, while hydrogen was liberated from the other hydride by means of the engine-cooling fluid. Considerable and sophisticated attention was paid to the engineering design of hydride beds for fastest possible charging and discharging for instance by G. J. Powers and D. C. Cummings of MIT. The kinetics of hydride formation or decomposition may be limited either by heat flow or hydrogen diffusion; the consensus appears to be that in the best designs, heat flow is limiting. Complete charging of a hydride bed in five minutes was claimed by one speaker; this is unlikely to be bettered.

Overall, the technology of hydrogen-fuelled motor cars seemed fairly promising; as several speakers pointed out, however, hydrogen-fuelled cars (like hydrogen as substitute natural gas) would probably require a huge discontinuous change in distribution over the whole of a large area all at once; it could scarcely come by easy stages.

Methanol, which can be made by a hydrogen route, received attention as a possible automotive fuel. In an impressive paper modified by some last-minute findings, T. B. Reed and R. M. Lerner of MIT dealt with the potential for progressive lacing of petrol with synthetic methanol, as methanol gradually becomes cheaper relative to oil. The prospects are promising, according to this work, that increased methanol content will so improve anti-knock properties that the lead content can be considerably lowered or perhaps even eliminated. The conflict of all-or-nothing versus progressive introduction of new fuels was one aspect of the 'social' approach to the hydrogen economy which several speakers essayed. Dickson's paper from Stanford was outstanding in this connection. He analysed the magnitude of the impact of various changes inseparable from a hydrogen-fuelled car economy and also examined which groups of people (drivers, mechanics, fuel dispensers, fuel distributors, fuel manufacturers) were affected. His histori-

Aviation will be the first major user of hydrogen, well before the century is out. The larger the aircraft and the greater the range, the greater is the economic advantage of using liquid hydrogen.

cal parallels—for instance, the disincentive to the spread of diesel-fuelled passenger cars which he identified with the mild inconvenience of locating supplies of diesel fuel—lead him to argue strongly that while quite major changes in engine design, such as the Wankel engine, can be quite readily accommodated, relatively modest changes in nature of the fuel, and especially of fuel distribution, will constitute major blockages.

One advantage of the kind of analysis offered by Dickson is that it can alert technologists to needs that otherwise might be underestimated or even ignored. If it is accepted that the introduction of a hydrogen-only car would come up against particularly intractable socio-political resistances, then there is a strong incentive to develop an engine that can run on either of two alternative fuels—such as the hydrogen/methanol engine described at the conference by

R. R. Adt of Greenwell and M. P. Swain of the University of Miami. Technology and economics are but two sides of a triangle, of which politics forms the base.

The long paper by T. C. Cody of West Virginia University on the hydrogen economy and (American) law was particularly thought provoking. Cody foreshadows in-

If it is accepted that the introduction of a hydrogen-only car would come up against intractable socio-political resistances, there is a strong incentive to develop an engine that can run on either of two alternative fuels.

creasing "federal preemption" over the states in safety and environmental legislation and a progressive sharpening of legal limitations on energy use: eventually he foresees a law of allocation to replace the present 'crazy-quilt of contradictions, cross-purposes, omissions, overlap and duplication.'

On hydrogen for aviation, opinions were also divided. R. A. Lessard, an economist of the United Aircraft Research laboratories, in a separately circulated paper, was sceptical on purely economic grounds, whereas P. F. Korycinski and D. B. Snow of NASA were optimistic on the basis of a detailed technical analysis. The larger the aircraft and the greater the range, the greater is the economic advantage of using liquid hydrogen. One listener at least was left with the clear impression that aviation will be the first major user of hydrogen, well before the century is out. This view was shared by a severe critic of the hydrogen economy, P. N. Ross, a vice-president of Westinghouse, who was utterly convinced that nuclear electricity, distributed as such, would indefinitely defeat the use of hydrogen—except for aircraft.

There is no space to do justice to the group of papers on solar and geothermal energy (the latter seems particularly promising in an American context). The conference showed very clearly its recognition that hydrogen is merely a means of storing energy, not creating it. Some new, cheap solar cells were developed and evaluated, the engineering aspects of a mile-square servo-controlled mirror array and boiler tower were analysed, there was much to do about large floating platforms making use of thermal gradients in the oceans (a form of indirect solar energy) and wind power came in for some detailed analysis, not least in connection with a self-contained domestic system using only established technology, including energy storage by electrolysis and later use of the hydrogen. This intriguing study was by L. W. Zelby of the University of Oklahoma, where the wind is gentle but persistent.

Indeed, the wind of hydrogen and of change blew gentle, clean and persistent at Miami. The fact that hydrogen is almost pollution-free was much commented on (but see *Nature* for March 29, page 458); the name of the Environmental Protection Agency was much mooted and an International Association for Clean Energy was founded at the conference. There was the sniff in the air of American technology on the move; much more will be heard of hydrogen before long.

Fuel prices and the energy crisis

Fuel	Price before the energy crisis (\$ per million BTU)	Estimated 1974 price
Natural gas	0.25	0.70
Fuel oil	0.32	1.80
Coal	0.20	0.58

international news

EVERY year 7,000 people in Britain die from kidney disease. Five thousand of these die simply because a cure for the chronic form of the disease has not yet been found; the remaining 2,000 fall into the category that are likely to respond to a kidney machine or to a transplant, but they die because there are not enough machines or cadaver (donor) kidneys to go round. In the short term, a rapid turnover to transplantation permits the acceptance of more patients into dialysis units and so an immediate and urgent need is for many more cadaver kidneys.

Twenty-three transplant units have been set up in the United Kingdom since the Medical Research Council advised the Department of Health and Social Security in 1967 that renal transplantation had 'passed beyond the research stage'. Surgeons now perform more than 500 transplants between them every year; the survival rate has continued to climb steadily and kidneys have proved themselves the most suitable of all transplanted organs. But the demand for kidneys far exceeds the supply, and it is a lack of understanding and cooperation among doctors themselves that is largely to blame.

"Organ donation" said a report issued by the Royal College of Physicians (RCP) in 1972 "involves the doctors caring for the patient in a considerable amount of extra work and trouble. They must talk to the relatives, consult the coroner and make numerous telephone calls. Most of these patients are not now considered as organ donors because the question is never raised. Yet this negative attitude is the major limiting factor in the expansion of a renal transplant service . . . Most important of all," the report concludes, "is for there to be more cooperation within the medical profession so that the donor organs are not wasted."

Some London hospitals were already engaged in a kidney-sharing programme and as a result of their example and the RCP's report, the Department of Health set up in 1972 the National Organ Matching Service based at the Southmead Hospital in Bristol. The names of patients waiting for kidneys are kept on a computer and when donor kidneys become available the most suitable recipient is selected. The following year, the Department issued 2,000,000 donor cards, which although never intended as legal documents were designed to enhance the public's aware-

ness of the kidney problem. But neither the donor cards nor the matching service has provided the results everyone was hoping for.

There were no more kidneys available last year than in 1972; and surgeons are particularly disappointed at the comparatively small number of matches effected by the Bristol centre. But there

Transplants: the failing machinery

Robin Laurance



is special concern about the reluctance of some hospitals to share out the kidneys which are not suitable for their own patients. Last year, for instance, the Hammersmith Hospital received 38 kidneys from the pool but contributed only six. St Mary's Hospital took 31 and contributed 8; Queen Elizabeth's Hospital in Birmingham, on the other hand, received 38 and contributed 74.

In addition to an apparent lack of cooperation between the surgeons themselves, there is an even greater gap between the surgeons and the rest of the profession. Mr Anthony Barnes (the surgeon involved in a recent controversial transplant operation at Birmingham, said to have been started before the donor was dead) says the main problem is ignorance and that the majority of doctors simply are not aware that transplants are such a successful treatment for kidney failure. His

own success rate is very high and yet a consultant from another department in his own hospital walked through his ward recently and asked whether he had anyone still alive after a transplant. Mr Barnes spends every spare minute visiting hospitals in his area reminding doctors and surgeons about kidneys. But there are still a number of hospitals from which he has never even had the offer of a kidney.

A member of another kidney team recently accused doctors of being reluctant to ask relatives for permission to take kidneys from newly dead patients. Some doctors, he said, take it as a personal failure when a patient dies, but they should appreciate that a kidney could save another life. "I know it seems callous," he said, "but we have to educate our accident colleagues that it is necessary to act quickly when a suitable patient dies."

Michael Bewick, who transplants kidneys at Guy's Hospital, London, says it is "quite appalling" to have to ask relatives for permission to retrieve kidneys soon after bereavement. He says the Department of Health should introduce an 'opting in' system where everyone would be formally asked to consider becoming a potential donor. The tissue details of all those who agreed would be kept on the computer at the Organ Matching Centre and when anyone died suddenly or was suffering from a terminal condition, the doctor would simply check if his patient was on the computer's list.

Roy Calne, Professor of Surgery at Cambridge, favours an 'opting out' system whereby everyone becomes a potential donor unless he registers his dissent. Professor Calne says a change in the law and more collaboration within the profession are the best ways of meeting the ever increasing demand for kidneys. "But", he says, "I doubt whether there will be a significant improvement until the public puts pressure on both the government and the medical profession. And that won't happen until the results of kidney transplantation are well enough known."

The Department of Health and Social Security is unlikely to introduce either an 'opting in' or an 'opting out' system in the near future, although Dr. David Owen, Under Secretary of State for Health, said recently in a Commons written reply that such a scheme was under consideration. The department is, however, launching a publicity cam-

paign with 30,000 posters to be sent to hospitals and doctors' surgeries reminding people that when they die, their kidneys could save other people's lives. But without a change in the law, any positive response will only cause frustration all round.

So the problem comes back to the doctors themselves, who have to make the most of the kidneys that come their way. One of the headaches here is defining the moment of death in the donor—a problem that has become even more difficult in recent months with the increasing use of ventilators. For a successful transplant, a kidney must be extracted within an hour of death and where the patient is being kept 'alive' artificially by ventilation the doctor (and in transplant cases, two doctors are required to certify death) must decide when the brain has died and when the ventilator has passed the point at which it will invoke any involuntary response in the patient.

Anthony Barnes maintains that many doctors are still only learning when to turn the ventilator off. Some, he says, do an injustice to relatives by allowing their patients to rot with a brain that is never going to work again. "And the kidneys become rotten too," he says, "and that's an awful waste." And to rub in the dilemma facing doctors, the coroner at the Birmingham inquest involving Mr Barnes, told the jury that



A patient receives a transplant kidney at Guy's Hospital, London

there was "considerable dissension among pathologists he had questioned about when a person was dead".

Doctors abroad involved in kidney transplants already have fewer problems. In France, and in some parts of the United States, brain death is accepted as a clear index of death, which means that much fresher kidneys are available from donors who are still breathing. (It also, of course, means

that many patients are spared lives as cabbages.) The Americans also have the Uniform Anatomical Gift Act which has made donor cards legal documents.

And in Scandinavia the law says a doctor can take what he likes from a corpse, with no questions asked. Clearly, legislation along these lines would make life easier for transplant surgeons working in Britain.

THE pay of government scientists in Britain will continue to be determined by reference to that of scientists in industry rather than by comparisons with other branches of the Civil Service if the recommendations of the Pay Board on Science Group pay are accepted as policy.

About 16,000 members of the group are affected by the report of the board, which appeared after a protracted dispute over the criteria for determining their salaries. The scientists, represented by the Institution of Professional Civil Servants (IPCS), were loth to accept a pay research exercise referring to the rates of scientists in industry because, it was argued, the government was such a large scale employer that industrial rates were themselves determined by the level of pay in the public sector, and so any comparison would be prey to an illogical circularity.

The report says, however, that the process of pay research is not invalidated by these special circumstances, and that they can be taken into account, either by the Pay Research Unit (PRU) in making comparisons or where this is not possible, by the parties involved in negotiation.

The IPCS had pointed out in evidence to the board that scientists

Pay Board reports on scientists

had not had a substantive pay review since January 1971, when there was a measure of parity between their rates and those of comparable grades in the Civil Service Administration Group. By January this year the scientists had fallen behind, at the scale maxima, by as much as £880 at Principal Scientific Officer (PSO) level, and £731 at Higher Scientific Officer level.

According to the IPCS new rates should have been determined, according to a principle of the Priestley Commission, by internal relativities, using the Professional and Technology Group as a comparison, rather than the Administration Group, as in the past. But the only point at which the Pay Board leaned towards internal relativities was in its suggestion that PSO rates should at both ends of the scale (currently £3,715 and £4,895) be no more than 5% away from the rates for their counterparts in the administrative grades. As a corollary, the report recommends that the maximum of the Senior Scientific Officer scale (now £3,895) should be adjusted

if pay research does not produce an appropriate differential with the minimum of the PSO scale.

Both these adjustments would take into account what the report calls the "unquantifiable" factors of individual merit and the differing career pattern of government scientists, who tend to go on working in research and development long after the age at which their industrial counterparts have moved on to greener pastures. About 5,500 scientists would be affected by this suggestion.

The remaining 10,500 members of the Science Group will have to be content with pay research, although there is a recommendation that procedures for the exercise should be reviewed jointly by the scientists, the Civil Service Department and the PRU.

The IPCS said it was "severely disappointed" with these formal recommendations, which would take a long time to implement (January 1976 has been suggested as the earliest date by which the exercise could even get under way). But the union adds that, given goodwill on the part of the government, there is no reason why an agreement should not be reached and it has entered an interim claim, based again on internal relativities.

Berkeley's line on PWRs

Eleanor Lawrence

THE Nuclear Laboratories at Berkeley in Gloucestershire provide the Central Electricity Generating Board (CEGB) with nuclear research facilities and expertise without parallel in any electricity utility in the western world according to CEGB Board Member Mr Donald Clark. At present, with the CEGB's controversial proposal to buy American-designed pressurised water reactors (PWRs) very much in the air, evaluating and verifying work on the safety of these reactors is one of Berkeley's most pressing problems.

According to Dr Bryan Edmondson, the director, very recent work by Westinghouse on the PWR pressure vessels indicates that there may be less danger than feared of the catastrophic crack propagation emphasised by opponents of PWRs. At the temperatures at which the reactors operate, defects in the steel pressure vessels would probably propagate by plastic deformation and not by sudden crack propagation. In a half-size model with 6-inch walls (compared with 12-15-inch walls in the full-size vessel) Westinghouse tests have shown that this does in fact lead to a 'leak-before-break' situation.

Dr Edmondson emphasises that this, although comforting, is not the sort of information on which the safety case for the reactor could be based. Quality tests during manufacture, overpressure testing before service and regular in-service inspections are the best lines of defence. Also, as the vessels need not be fully pressurised until a temperature of 300° C, they can be carefully controlled during the heating up process through temperatures at which the danger of catastrophic crack propagation is greatest. The pressure vessels are also more accessible to repair and inspection than the innards of the gas-cooled reactors and, as technology has been developed to deal with crack detection in those circumstances, Dr Edmondson is hopeful that it will not prove too big a problem with PWRs.

Commenting on the memorandum sent to the Select Committee on Science and Technology by Sir Alan Cottrell, highlighting the problems of pressure vessel safety, Dr Edmondson says that he could not disagree with Sir Alan and had been giving similar advice to the Board, but that the results of the Westinghouse tests had not been available then.

Berkeley is also evaluating the American calculations on what would happen to the pressure vessel in the case of a loss-of-coolant accident when it would be subjected to a shock of cold water.

The laboratories are now half-way through the stress analysis and so far have come up with the same reassuring answers as the Americans, whose calculations have been accepted in the safety case in the United States.

Dr Edmondson emphasises that because the PWR matter is so controversial much more evaluation and verification of already published results, which would normally be taken on trust, is having to be done. But he does not expect that the findings will differ drastically from previous work.

Russia's Mars probes

from our Soviet Correspondent

THE ANNOUNCEMENT of the partial failure of the latest Soviet Mars experiment—the probes Mars-4 to Mars-7—was one of the less auspicious features of the Jubilee General Meeting of the Academy of Sciences on March 5th 1974. Already it was known that the breaking motors of Mars-4 had failed to operate so that it achieved only a close by-pass at 2,200 km. Nevertheless some good photographs had been transmitted from Mars-4, and Mars-5 was satisfactorily in orbit investigating the planet, its atmosphere and ambient space and two further probes were still on their way.

However, Mars-6 and -7, which reached the vicinity of the planet on March 12th and 9th respectively were unable to retrieve the hopes of the programme. Although the official Tass announcement called their flight "an important step in the investigation of Mars", the news was depressing. The two probes had carried descent capsules, which would investigate the surface and relay data to the orbiting section for re-transmission. There had even been speculation (evoked by a Pravda article on future plans for planetary research) that one of them might be carrying a roving "Marsokhod". However, radio contact with the descent stage of Mars-6 was lost when it had reached "the immediate vicinity of the surface", and a failure in the on-board systems of the descent stage of Mars-7 after separation resulted in a fly-by at 1,300 km. The "orbital" stages of these two probes apparently failed to enter aero-centric orbit, since they are reported to be "investigating the physical characteristics of space" from helio-centric orbits. So the overall design of the experiment, with four orbital stations monitoring the same data from different altitudes together with two surface probes (the previous programme used only two such stations and one surface probe), has been reduced to a single orbital station and a number of

fly-by photos, valuable though these are.

The claims in the Soviet press that nevertheless valuable results have been obtained are not however merely an attempt to save face. Photometry readings, for example, have revealed a maximum precipitation of some 60 nm—a value several times greater than that recorded by similar instruments aboard Mars-3 in 1972, which, it is suggested may indicate a different rate of release of water vapour from the crust into the atmosphere in different regions of the planet. Traces of atmospheric ozone have been noted for the first time. The existence of the magnetic field of the planet (indicated by the data of Mars-2 and -3) has been confirmed, and readings have now been obtained for the magnetic field on the dark side of the planet.

Nevertheless, a sense of disappointment is apparent and a long article in Pravda, March 20th, on the engineering problems of designing planetary probes may well reflect not only a desire to restore public confidence but also some serious re-thinking by the planners of the "hardware" situation.

ELSE in Israel

Nechemia Meyers, Rehovot

EDITING and publishing a scientific journal is a very ordinary venture which anybody can do, and almost everybody does—at least, in the opinion of Mr Paul Nijhoff Asser, Secretary General of the International Group of Scientific, Technical and Medical Publishers. Mr Nijhoff Asser made this judgment at a recent meeting in Israel of a small but influential group of scientific communicators, the Executive Council of the European Association of Editors of Biological Periodicals (ELSE). He was commenting on a report from ELSE treasurer, Miriam Balaban, that 130,000 scientific journals are currently being produced and that the number is rising.

The European editors, who in the past have seemed anxious to demonstrate their independence of United States influences—preferring, for example, to issue their own manual of scientific writing and terminology—nevertheless invited an observer from their American counterpart organisation, the Conference of Biological Editors. Dr Karl Heumann, Executive Editor of the Federation of American Societies for Experimental Biology, took an active part in the discussions and suggested that editors should carefully consider whether to publish papers describing experiments in which the human participants were not aware of the risks they were taking.

Small though the meeting was, the Israelis present regarded it as another victory in their continuing struggle to establish their European credentials.

Academy exposes a sham

Colin Norman, Washington

FOR years now, spokesmen for the Nixon administration have been touting the line that federal research and development expenditure are gradually being focused on programmes which will benefit society and help solve "critical domestic problems" in the United States. Such sentiments reached their zenith two years ago, in March 1972, when President Nixon sent his first, and only, message to Congress on science and technology. His statement was replete with rhetoric about a new partnership which would be forged between government, industry and the universities, to help bring the fruits of technology to the people.

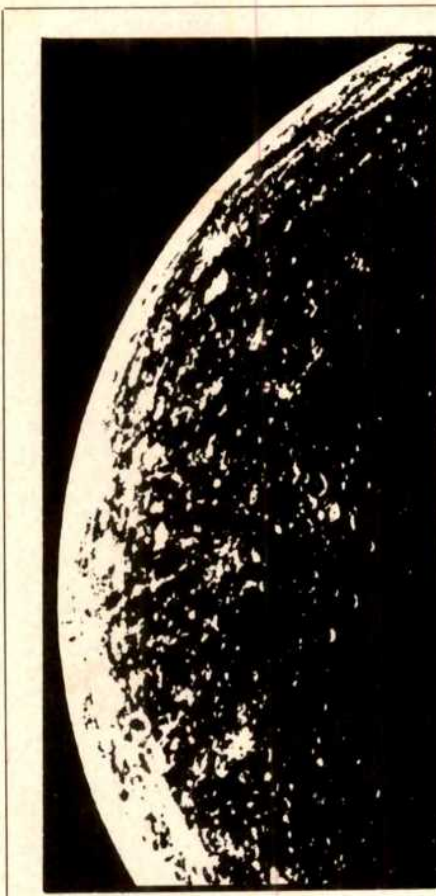
But a committee of the National Academy of Engineering (NAE), the most prestigious engineering organisation in the United States, published a report last week which came close to suggesting that most of President Nixon's science and technology message has turned out to be a complete sham.

The committee states that "with a few exceptions, the vast technology developed by federally funded programs since World War II has not resulted in widespread 'spin offs' of secondary or additional applications of practical products, processes, and services that have made an impact on the nation's economic growth, industrial productivity, employment gains and foreign trade".

The committee's general thesis is that technology developed with federal funds probably hasn't been exploited for the good of the people (or at least for the good of corporate profitability) because "a plethora of structural and institutional barriers exist in the federal government and the private economy to prevent the efficient and effective utilisation of this technology". Furthermore, since there has never been a comprehensive effort to search out technology which can be usefully exploited, it is difficult to recommend policies in such a vacuum of knowledge.

The NAE's findings and conclusions are shared by many observers of federal science policy, including a few key members of Congress. It was only just over a year ago, for example, that the Senate passed, by a huge majority, a bill sponsored by Senator Edward M. Kennedy which would have given the National Science Foundation a large budget to bring federal research and development to bear on such critical problems as urban renewal, health care delivery and transportation.

The bill died because it was never approved by the House of Representatives, but the sentiment lives on. Just



MERCURY as seen by the cameras of Mariner 10. Left, heavily cratered area reminiscent of the highlands of the Moon, including a bright rayed crater. Picture taken from about 400,000 km resolves craters 120 km or more in diameter. Below, closeup picture taken from a distance of 20,700 km about 30 min before closest approach. The clearly defined central crater (about 12 km across) is a relatively new feature.



last month, for example, the Senate Committee on Science and Astronautics held extensive hearings on a bill which would provide NASA with a large helping of money to promote civilian technology. The bill is not likely to get anywhere this year because, even if it is approved by the Senate, the House of Representatives has so far shown little interest in the measure and in any case congress is likely to be occupied later this year with impeachment fever.

But there continues to be considerable feeling on Capitol Hill that there is a lot of federal research and development which could be usefully exploited if only suitable arrangements could be worked out to accomplish the task.

The NAE committee, at least, has a few concrete recommendations for removing some of the barriers to innovation which have prevented private companies from turning federally produced technology into marketable products. For a start, it suggests that the federal government should spend \$1,000 million a year in helping to promote the utilisation of technology through a variety of incentives and government programmes.

The first task should be to conduct a survey of federal laboratories, to test "the assumption that there is a substantial amount of useful federally funded technology available for beneficial, widespread secondary application."

Assuming, as the committee does, that

the survey turns up positive results, federal agencies should set up explicit programmes with specific charters for embarking on technology utilisation projects, and they should be given funds earmarked in the agencies' budgets for that purpose. Certainly, some agencies, such as NASA, have already established such offices, but the NAE committee reckons that much more could be done. But the committee is quick to point out that its recommendations in no way "imply that the federal government should become a competitor to the private entrepreneur", for the idea is that once a technology has been developed, it would be adapted, produced and marketed by private industry.

In order to stimulate that end of the process, the committee suggests that the federal government should provide a variety of incentives such as risk assurance, exclusive licences and "imaginatively bold financing to users in the public and private sectors in order to accelerate the direct implementation or to stimulate financial institutions to provide greater investment in new technology".

If all that sounds familiar, it is. Such suggestions have been made by virtually every committee which has studied the federal government's role in exploiting research and development financed by the taxpayer for the good of the taxpayer. But so far, there's little sign that anybody is listening.

correspondence

Newton and Kepler

SIR,—I have only just read Dr J. W. Herivel's review in your journal (*Nature*, January 18, 163–164) of the recently published variorum edition of Newton's *Principia*. There I am fathered with I. B. Cohen's view—"originally put forward [in my article on 'Newton's early thoughts on planetary motion in'] *Br. J. Hist. Sci.* 2, 117–137 (1964)"—that Newton "apparently learnt of Kepler's law of areas in 1678 and at once was able to solve the problem of Keplerian planetary motion" (Herivel's italics). Quite bluntly, I have never said anything approximating to that, with or without its emphasis, there or anywhere else.

In my 1964 article I did, to be sure, publish my (then) novel finding that nowhere in his extant early papers "does Newton make any mention of Kepler's area law"—with the qualification in a footnote thereto (page 124) that in his "deep study at an early age of Wren's . . . tract [on Kepler's problem, as published by Wallis in his *De Cycloide*, 80 (1659)] . . . it is just possible his attention was caught by the fleeting reference [there] to the area law"—and then went on to observe (page 131, note 48) that Newton possessed in his personal library a "well-thumbed" copy of Nicolaus Mercator's *Institutiones Astronomicae* (1676) where, as I wrote, "correct enunciation" of the planetary law is given. That it is, as Herivel now says, "stretching credulity to suppose that Newton had not . . . deeply pondered on this extraordinary law before 1679 [sic]" may or may not be everyone's modern reaction; but such an undocumented opinion is certainly irrelevant when, as I did, one comes to examine the available historical evidence. I know nothing of Herivel's recent scholarly activity in this area but after my own nearly twenty years of continuous study of Newton's scientific papers (including many in private possession which are not generally accessible) I will reiterate that I have as yet found nothing which establishes in any way that Newton was, before 1676, aware of the law's verbal enunciation or begins to hint that he "deeply pondered" its meaning at any time before late 1679. If Herivel knows better than I, let him state his sources.

As for the portion of Herivel's quot-

ation which he italicises, this, whatever its truth, greatly traduces the hypothesis which I formulated in my 1964 paper. There I made Newton's "sudden" appreciation of the validity of the area law in 1679 dependent, not on his presumed ignorance of the law's statement up to that time, but on his recognition of its theoretical necessity in his proof of the elliptical orbits of the planets under the condition (as Hooke laid it down in their correspondence in the early winter of 1679–80) that their undeviated motion be uniform and in a straight line. Herivel knows this very well since, on the appearance of my article 10 years ago, he mounted a hasty and often ill-considered but full-blooded attack on it in *Br. J. Hist. Sci.* 2, 350–354 (1965), in the course of which he allowed my hypothesis to be "possible [but] very improbable". That unsupported judgement is evidently now to be sanctified by similarly ruling out

Brevity

CORRESPONDENCE stands more chance of being published if it does not exceed 350 words. The Editor reserves the right to abbreviate any item.

as "very improbable" a distortion of my original thesis which I have never held. May I ask Herivel to acknowledge as much and to retract his present aspersions?

D. T. WHITESIDE

Cambridge, UK

DR HERIVEL REPLIES: Dr Whiteside protests at my "fathering" on him the view of I. B. Cohen that Newton "apparently learnt of Kepler's law of areas in 1678 and at once was able to solve the problem of Kepler motion" (my italics). This view seems however, to have been suggested to Cohen by Whiteside's 1964 paper to which he refers immediately afterwards. This does not seem so improbable as it might otherwise appear from Whiteside's letter in the light of the following passage in his paper (page 128):

"We have said enough to justify our general contention that in the autumn of 1679 Newton was, if indeed he at all consciously then recognised its existence, still unwilling to allow Kepler's crucially important second (areal) law even an empirical place among the axioms of his astronomical thought."

I myself derived somewhat the same

impression as Cohen from a reading of Whiteside's paper. For in my "hasty and often ill-considered" paper of 1965 I said:

"to the various explanations already put forward, a new one has recently been added by D. T. Whiteside, namely, that Newton was prevented from solving the problem of Kepler motion before 1679 because up to that time he was either unfamiliar with, or had no confidence in, Kepler's second law of planetary motion".

As regards the second, italicised, part of Cohen's view, although I did not draw this conclusion myself I can quite understand Cohen's doing so. For if Whiteside's paper is interpreted as implying that Newton was unfamiliar with Kepler's second law up to the autumn of 1679, and if he required this law for his solution to the problem of Kepler motion in the following winter, then it does not seem unreasonable to conclude that it was his tardy acquaintance with this law which led to the solution of the problem.

In the light of Whiteside's letter I am quite happy to retract the phrase "This view originally put forward by D. T. Whiteside". But in fairness to I. B. Cohen, and for the sake of the historical record, I should want to replace it by the following: "this view was apparently suggested to I. B. Cohen from a reading of a paper by D. T. Whiteside".

As to the important question of whether or not Newton was consciously aware of the exact, areal form of Kepler's second law before the autumn of 1679, it seems to me that in the light of Dr Russell's findings the onus is on Whiteside to provide documentary evidence for a lack of familiarity on Newton's part. In the absence of this evidence I for one will continue to believe that Newton was perfectly well aware of the exact form of the law long before 1679, most probably from a reading of Kepler's *Epitome*. For if Dr Russell is correct in believing that this famous work of Kepler was "from about 1630–50 or beyond almost certainly the most widely read treatise on theoretical astronomy in Europe" (*Br. J. Hist. Sci.* 2, 20; 1964) then I find it very difficult to believe that it was not read by Newton. Documentary evidence is important, and without it history cannot be written, but its absence does not necessarily preclude the historian from drawing probable conclusions in other ways.

news and views

Should fossil hominids be reclassified?

RICHARD LEAKEY's report in this issue of *Nature* signifies the end of the sixth field season in the Lake Rudolf area. In those six seasons important new fossil material has appeared at a rate almost too fast for scientific interpretation if not intellectual assimilation. At present, it is possible to discern two important contributions of the East Rudolf work, although a greater time perspective may indeed add further dimensions and ramifications to present interpretations of the Rudolf material.

The first and most obvious contribution has been the enormous increase in the size of the fossil sample, not only of hominid material but also of other Primates and vertebrates. The work at Rudolf has so far yielded approximately 107 specimens of fossil hominid; by comparison, Olduvai Gorge, an area noted to be very productive of fossil material, has yielded only about 40 hominids in nearly as many years. A second important result of the Rudolf work has been a recognition of the necessity to examine closely some of the earlier concepts and interpretations of human evolution. Before the 1970s the widely accepted model of human evolution suggested a slow and orderly emergence of a more advanced evolutionary grade of man from a primitive basal stock. In this way, late Pliocene-early Pleistocene australopithecine populations were seen to develop slowly into middle Pleistocene populations, generally attributed to *Homo erectus*; in fact, the process may not have been so simple.

The fossil evidence of man at East Rudolf now demonstrates a very wide morphological variation. There is clearly a large, robust-type australopithecine as seen in the cranium KNM-ER 406, mandibles KNM-ER 729, 818, 1806 and other specimens. There is also evidence of a more lightly built and perhaps more advanced group seen in the crania KNM-ER 1470 and possibly 1590, mandibles KNM-ER 730, 820, femur KNM-ER 737 and others. Certain specimens, notably the cranium KNM-ER 1805 and the mandible KNM-ER 1482, have proved difficult to assign to any currently recognised group. An interesting aspect of the Rudolf material has been the apparent absence of the gracile form of australopithecine. This type is well known from sites in South Africa and from Olduvai Gorge and now Leakey suggests that a cranium recovered during the last field season, KNM-ER 1813, may indeed represent that group at Lake Rudolf.

The variety of morphology present in the Rudolf hominid sample poses a number of interesting questions and of these perhaps the most important concerns the relationship between the advanced primitive members of the gracile groups. Does the gracile group contain generically distinct *Australopithecus* and *Homo* or does it contain only one taxonomic group with wide variation due to temporal, behavioural, sexual or geographical factors? Robinson, in placing all of the more gracile types in the genus *Homo* (*Nature*, 205, 121; 1965), has favoured the second explanation. His classification, based largely on features associated with dental mechanisms, implied significant dietary differences between the robust and gracile groups. Leakey now suggests that there may be two gracile groups—one advanced and one with more primitive morphology, distinct at the generic

level—in addition to a large, robust group and perhaps one other. He thus suggests the existence of at least three separate hominid lineages, sympatric and contemporary, within the early Pleistocene. Such a classification imposes problems.

First, it is difficult to understand how the East African environment could provide adequate ecological space to support three or more separate hominid lineages which must have been in competition for territory and for some basic subsistence materials during a period of 2 million years or more. Leakey's interpretation of the evidence would seem to suggest an early adaptive radiation within the Hominidae but since all groups were living in presumably the same environment, it is difficult to visualise the adaptive pressures and isolating factors which are necessary to produce genetic separation in related groups. Finally, and most important, if one accepts that gracile australopithecines and *Homo* coexisted in the early Pleistocene, then one must also accept that the available sample of australopithecines does not, and indeed cannot, represent the ancestral hominid group. The gracile australopithecines are widely accepted as the basal hominid stock and yet Leakey's current classification of the early hominids implies that the known members of this group coexisted with the genus *Homo* and were in fact too late to provide the ancestral population.

The problems associated with interpretations of the Rudolf material demonstrate the urgency of the need to re-evaluate the taxonomic criteria for genera within the Hominidae. The definition of the genus *Homo*, proposed in 1964 by Louis Leakey, Tobias and Napier (*Nature*, 202, 7) is inadequate on several grounds and failed to demonstrate adequate morphological space between the gracile australopithecines and *H. habilis*, the most primitive member of the genus *Homo*. Robinson's classification separated the robust australopithecines ("*Paranthropus*" in his terminology) from all gracile hominids ("*Homo*") on the basis of dietary and behavioural factors. Still others have suggested that all of the australopithecines should be included within a single taxa. There is still, however, no widely accepted classificatory system. The present difficulty is due partly to the inevitable incompleteness of the fossil sample and partly to the inadequacy of Linnaean systematics in dealing with evolving populations; in the particular case of the Rudolf material, Leakey's typological approach to classification adds yet another dimension to the difficulties. Palaeontologists must be dealing with evidence of evolving lineages and yet by continuing to compartmentalise this evidence into discrete taxa based on typologically orientated criteria, they are losing sight of the continuity and cohesiveness of the fossil record. Typological classification emphasises the distinctiveness of the material and must, also, therefore, implicitly suggest discontinuity in the fossil record. A more appropriate approach might emphasise functionally-based similarities and expected variability in evolving populations and would emphasise the continuity of the evolutionary process.

Leakey suggests in this issue that in view of the large amount of fossil human material now available he intends to make a review of hominid systematics. Such a review is necessary, but before it can be of practical importance, the theoretical bases of hominid palaeotaxonomy must be re-evaluated and if necessary reformulated. Without a valid and widely acceptable theoretical framework for classification of the fossil Hominidae such a taxonomic review may be premature and will only add further controversy.

From our Palaeoanthropology Correspondent

Predicting how proteins fold up

In current and pending publications there are signs of a new, more ambitious phase in the struggle to predict the biologically active conformation of a protein from its covalent structure. Certainly there is much incentive for ambitious investigations in this field. In the right conditions, many proteins spontaneously and reversibly fold into their biologically active conformation maintained by intramolecular non-bonding forces and reducible disulphide bridges. Such observations have appropriately been interpreted as evidence that the linear sequence of amino acid residues which comprises the covalent structure of the protein carries all the necessary information for directing the folding process, and it is just a short step to speculation that artificial synthesis or genetic engineering of novel sequences will lead to conformations with novel catalytic and control functions. But which sequences will lead to the desired conformation, and achieve it in reasonable time?

The latest contenders in pursuit of the answer are Ralston and De Coen (*J. molec. Biol.*, **83**, 393; 1974) and Nagano (*ibid.*, **84**, 337; 1974). Both follow current opinion in seeking the answer in nucleation, that is, in centres of conformational structuralisation which initiate and guide the subsequent folding up of the protein molecule.

Ralston and De Coen carry out conformational energy calculations on a variety of possible nucleating structures of varying complexity, though generally on sequence fragments of six or less residue units. Principally, the object of the exercise is to find the favoured (lowest energy) conformation for a variety of short sequences. In order to reduce the computation to reasonable proportions, some severe approximations are made. In particular, these workers exploit the Liquori concept (*Q. Rev. Biophys.*, **2**, 1; 1969) of a stereochemical alphabet in which only a small number of specified conformations is allowed for any amino acid unit and interactions between units merely determine which of these is the favoured one. Furthermore, not all sidechain conformations are adjusted in the search for the lowest energy confirmation, and neither are geometric variables other than rotations around single bonds. A true minimisation of the energy of the overall system could yield rather different results, and it is surprising that these workers do not apply established minimisation procedures even where these could be easily and fruitfully applied. Nevertheless, interesting conclusions are drawn concerning the favoured conformations of many short sequences which could, within much longer sequences, present the first appearance of structural organisation during the folding process and so act as nucleation sites.

It could be argued, however, that work of this kind is premature. Perhaps another, less ambitious phase should properly precede this one—the phase of consolidation of the methods used to calculate the conformational energies. Conclusions concerning nucleation are likely to be no more credible than the least reliable conformational energy function used. Complete substantiation of the results of Ralston and De Coen awaits full minimisation and the advent of more realistic conformational energy functions, or at least the substantiation of those already in use.

For this reason the calculations of Nagano would seem to be on safer ground. Nagano belongs to the school of investigators who predict nucleating structures not on the basis of conformational energy calculations but on the basis of statistical analysis of proteins of known sequence and conformation. A crude example of this type of approach might be the observation that glutamic acid tends to begin a run of residue units in an α -helical conformation, and

therefore it might be predicted to do so in a protein of unknown conformation. The approach of Nagano, however, follows current trends in being much more sophisticated and quantitative, and takes account of the effect of pairs of residue units at different separations along the sequence. In his current paper, Nagano reports on the predictability of α helix, extended chain regions which are potential candidates for β -pleated sheet structures, and looped conformations of the backbone, in proteins of recently determined conformation which do not appear in the data of the initial statistical analysis. How good are the predictions for new proteins of completely unknown conformation or when the conformation, if known, is not taken into account.

Nagano reports that the accuracy of the predictions "seems to depend very strongly on the actual data used", and concludes that his predictions are less reliable for the new proteins. The predictions of α helix are good, however, providing the protein is not rich in β -pleated sheet, and the predictions of β -pleated sheet are good if the protein is not rich in α helix. The 'goodness' of a prediction is assessed in terms of a number of different accuracy statistics.

The results reported are at first glance disappointing to those who have learned from past publications in this field to expect steadily increasing degrees of accuracy. The important point is, however, that Nagano goes on to show that predictability can be enhanced by taking into account interactions between α helix, potential β -pleated sheet and loops. In particular, the consequences of β -pleated sheet structures occurring in the vicinity of α helices are considered. Nagano is therefore taking the first really serious look at the role in the folding of proteins of interactions between nucleating structures. Connoisseurs of the art of predicting protein conformation may feel that there is nothing entirely new in his reasoning, but at the very least Nagano presents a comprehensive preview of the problems to be encountered.

Nagano concludes his analysis of nucleation with a brief discussion of the final stage of protein folding after the topological pathway of the protein backbone in three-dimensional space is roughly determined. Recognising the importance of the close packing of the hydrophobic sidechains during this stage, he would do well to consider the recent papers of Richards (*J. molec. Biol.*, **82**, 1; 1974) and Wishnia and Lappi (*ibid.*, **82**, 77; 1974). Richards presents the most detailed analysis so far of the tight packing of atoms in proteins of known conformation, and he concludes that simple geometrical packing may provide useful criteria in guiding and evaluating trial structures in simulations of protein folding. Wishnia and Lappi, on the other hand, have investigated experimentally the binding of apolar ligands to hydrophobic sites, using the interaction of cyclohexane and n-heptane with α and β -cyclodextrins as a model. If one considers the final stages of the folding of a protein as a folding of hydrophobic sidechains onto a previously established structure with a hydrophobic surface, then it is clear that work of this type will play an important part in providing thermodynamic parameters for the simulation of such a process.

What does seem clear is that the final stages of folding will be the most difficult to consider theoretically and therefore to predict. For simulating this stage of folding, the concepts and parameters obtained through investigations like those of Richards, Wishnia and Lappi will be necessary but not sufficient. The number of conformations to be treated and compared is clearly astronomical, and success will owe as much to the size and speed of future generations of computers as it does to the programs and data fed to them. But it is remarkable that one can now discuss the difficulty of predicting the final stages of folding, not the folding as a whole. This position has been achieved because of workers like Ralston, De Coen and Nagano who have done much towards reducing the number of initial and intermediate conformations in the folding process.

B.R.

Towards improved ways of localising tumours

THERE are many current investigations aimed at improving methods for localising both primary and metastatic tumours. Non-specific radio-labelled proteins, for example, have been used by some investigators (Spar *et al.*, *Cancer*, **20**, 865, 1967; Bonte and Curry in *Radioactive Isotopes in the Localisation of Tumours*, 80, Heinemann, 1967). Recent findings in experimental and human tumour immunology have, however, generated renewed interest in the possible use of specific reagents, namely radiolabelled antibodies, as a novel type of localising agent.

The success of this approach will depend, among other considerations, on the specific activity of the labelled antibody, its specificity for the tumour cells and on tumour site, cell composition and size. Although little success has been claimed for such studies using some experimental tumour systems, specific and significant *in vivo* localisation of labelled antibodies against a tumour-associated transplantation antigen of a methylcholanthrene-induced rat tumour was attained by Izzo *et al.* (*Proc. Soc. exp. Biol. Med.*, **139**, 1185; 1972).

The carcinoembryonic antigen (CEA) is a human oncofetal antigen associated with the glycocalyx of tumour cells, and is released into the plasma and other body fluids. It was originally considered to be associated solely with endodermally-derived carcinomas (Gold and Freedman, *J. exp. Med.*, **122**, 467; 1965); further studies have shown that CEA, or 'CEA-like' materials, are released into the body fluids from a wide variety of neoplastic and some non-neoplastic conditions (see, for example, *Br. J. Cancer*, **26**, 335; 1972). Last year, Primus *et al.* (*Cancer Res.*, **33**, 2977; 1973) reported that a mucus-secreting carcinoma growing in immunologically-depressed hamsters and derived by serial propagation in hamsters from a human

colonic carcinoma, exhibited preferential uptake of radio-labelled heterospecific anti-CEA antibodies. In this week's issue of *Nature*, Mach and his colleagues now report similar findings for a human colonic carcinoma growing in nude mice and that this antibody accretion may be detected by suitable external scintillation scanning equipment. Unfortunately, in both studies, the labelled antibody was not located specifically in tumour tissues and there was a long delay after its administration before optimal tumour content of the label was attained.

Iodine is not the ideal scanning isotope; so far, it has only yielded labelled antibody with a relatively low specific activity. Isotopes with superior scanning properties such as technetium-99m may give better results, although the initial rate of isotope accumulation will be important in determining the usefulness of the method.

Other practical limitations include the presence of significant amounts of circulating antigens which may bind the heterospecific antibody and the presence of appropriate autoantibodies on the tumour cell surface. Both phenomena will serve to decrease the amount of labelled antibody eventually located in the tumours. The studies of Primus and Mach and their colleagues have been carried out in optimal conditions of tumour site and type, yet the smallest detectable lesion weighed 200 mg and was located superficially. Moreover, the presence of extensive tumour necrosis was also a limiting factor which, when considered with the problem of circulating antigen excess, reveals that there are many difficulties to be overcome before hepatic metastatic lesions of colorectal carcinomas with their characteristic central necrosis will be visualised in this manner in patients.

Consequently, this approach to tumour localisation does not have immediate clinical applicability. It does, however, reveal another possible area of fruitful investigation which may eventually yield not only improved diagnostic aids but also therapeutic regimes through the use of antibodies, coupled to anticancer agents.

A.M.N.

Beyond systems ecology

WITH the increasing disturbance of natural systems by modification of the environment, the need for a way to predict the behaviour of complex ecosystems is rapidly becoming paramount. Because of the scale of the problem, the study of ecology is moving away from simplified systems and the analysis of elemental interactions towards systems in which the numbers and kinds of interactions are not under control and cannot be isolated. Extrapolation from two- or three-population models to large scale systems is often inappropriate and can give drastically misleading results.

The first attempts to derive a predictive theory of complex ecosystems were based on techniques well known to engineers under the general heading of systems analysis. A systems model is defined in terms of elements, each of which has one or more inputs and outputs and is specified in terms of the relationships between the various inputs and outputs of each element. With this very broad definition virtually all models are systems models of some kind but the term is usually used in cases where the number of elements in the system is large enough for detailed analysis of each one to be impossible.

Systems models have been applied on a small scale to ecological problems for the past one hundred years or so, usually in agricultural pest control or wildlife or resource management. The emphasis in most of the earlier applications was on the factors which affect a single population, usually one of commercial importance. These systems models were successful and widely used but limited

in extent by the amount of calculation necessary to analyse them. With the development of high-speed computers, Watt of the University of California, Davis and Holling of the University of British Columbia, and others, recognised that systems models in ecology could be used on a larger scale. The logical basis for their approach to the problem is the same as in the simplest models, but the availability of computers made it possible to use the models to determine and use the input-output relationships for all the populations within a community rather than only one of them.

The systems approach to ecology seemed so promising and the need for useful predictive models so great that between 1964 and 1972 funding of participation in the International Biological Program (IBP) has been at the level—unheard of in ecological research—of more than \$40,000,000 per year. Ecologists, engineers, meteorologists and mathematicians were gathered for the study of several systems ("biomes"), such as deciduous forest, intertidal zone, short and long grass savannah and desert. Extensive measurements were proposed as the basis for equally extensive computer models whose value could be determined only by a full scale program.

One of the reasons for the appeal of the systems approach, particularly to those not directly involved in ecological research, is its apparent objectivity. Ideally a systems model could be constructed by somebody with no prior knowledge or intuition of the system he is studying. The relationship between the elements would become clear

after a sufficient number of measurements. Since one of the difficulties in understanding the behaviour of ecosystems is ignorance of potentially important interactions, the systems approach seemed to eliminate any bias by design.

Unfortunately the promise of systems ecology has not been realised. The accumulation of accurate measurements and the construction of the first systems models lead not to useful predictive models of the various biomes but a recognition of the need for more measurements and more elaborate models. The successful systems models reduced the scope and analysed some of the subsystems in detail. Two problems have contributed to the difficulties encountered in the IBP studies. First, although computers are becoming larger and faster, there are still finite limits to the amount of information which can be analysed. The number of potential interactions increases with the square of the number of elements and the addition of one new element to the model requires the measurement of its relationship with all previous elements. Although common sense can reduce the number of interactions without reducing the objectivity of the approach (mice do not directly ingest soil arthropods), the quadratic increase remains. Second, the uncertainty and error inherent in ecological measurements greatly reduces the utility of a large model. A 5% error in each interaction might not significantly affect the predictions of a three-element model, but could invalidate those of a twenty- or thirty-element model.

An alternative to the systems approach has been developed independently by Levins of the University of Chicago and May of Princeton (*Proc. N.Y. Acad. Sci.*, in the press). "Loop analysis", as Levins calls it, is essentially hierarchical and depends on the initial assumption that the interaction between two elements of the system can be determined to be positive, negative or zero. It is thus formally equivalent to analysis of matrices with entries of +1, -1 or 0. In some simple cases, many of the important properties of a system can be determined without knowledge of the strengths of the interactions; the signs are sufficient. Levins (*Stability and Complexity in Model Ecosystems*, Princeton University Press, New Jersey, 1973), describes some examples for which the stability of the system and the sign of response of one of the elements to perturbations of one of the other elements can be determined.

The potential utility of loop analysis is not limited, however, to such simple systems. Although the results for more complex systems may be indeterminate when the behaviour depends on the strengths of the interactions, loop

analysis can identify those interactions or combinations of interactions (loops), which are the most important in determining the behaviour of the systems and thus provides the technique for reducing drastically the number of measurements which are necessary for a predictive model.

There are, of course, limitations to this approach. There is a possible bias introduced by the assumed signs of the interaction terms. There is no objectivity in the initial specification of the loop diagram, so that it is dependent on the intuition of the researcher. This means that it is necessary to investigate the consequences of ignoring certain interactions before reaching any conclusions. Further limitation is that if most of the interactions are in fact important in determining the behaviour of the system, nothing is gained over the systems approach. When a ecological system is inherently complex, loop analysis cannot simplify it, but when the system has had some simple aspects (and many ecological systems do if viewed correctly) then loop analysis can often reveal the simplicity.

From a Correspondent

Narrowest room temperature Mössbauer resonance

from a Correspondent

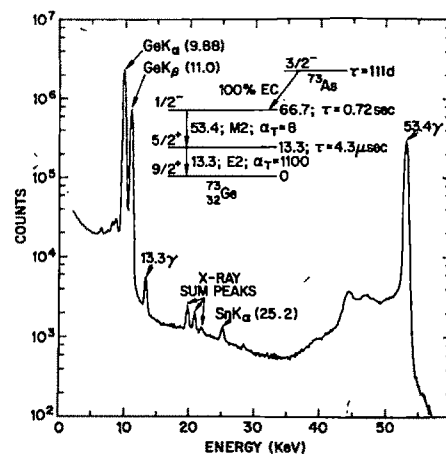
A RECENT paper by Raghavan and Pfeiffer (*Phys. Rev. Lett.*, **32**, 512; 1974) has shown that the Mössbauer effect can be studied using ^{73}Ge . The 13.3-keV transition to the ground state has long been one of the most attractive candidates for high resolution experiments because of the extreme sharpness of the resonance ($E\gamma/\Gamma_{\text{nat}}=10^{14}$ where $E\gamma$ is the Mössbauer γ energy and Γ_{nat} is the theoretical value of the full line width at half maximum of the nuclear transition), and because the low energy of the γ photon assures that a high probability of recoil-less transitions will result even at room temperature. The observation in this paper that resonance does occur indicates that a potential 28-fold improvement in $E\gamma/\Gamma_{\text{nat}}$ over the 14.4-keV transition in ^{57}Fe is now available. This constitutes a major advance in the search for a high resolution tool for use in physics, chemistry and technology of germanium.

The width of the absorption line reported is about seven times Γ_{nat} but despite this broadening it is the narrowest Mössbauer line observed so far at room temperature. This extremely sharp line could, theoretically at least, measure very small changes in the s-electron density, the electric field

gradient and the magnetic field at the Ge nucleus.

The paper does indicate, however, the severe experimental difficulties involved in the use of this isotope as a source of extremely sharp electromagnetic radiation. In the figure, the $^{73}\text{As} \rightarrow ^{73}\text{Ge}$ decay scheme, recently clarified by the use of high resolution Si(Li) detectors, is shown. The main problems which arise from this decay are, first, the high internal conversion coefficient, α_{IC} , of the 13.3-keV γ photon of 1,100 (that is, only 1 γ photon in 1,100 escapes from the ^{73}Ge atom without being internally converted into an electron); second, the close proximity of the γ -photon energy to the K-absorption edge of Ge ($=11.1\text{-keV}$); and, third, the low natural abundance of ^{73}Ge in natural Ge of 7.6%. Because of the first two problems the resonance cross section is smaller than the nonresonant electronic absorption cross section in Ge at 13.3-keV ($\sigma_{\text{res}}/\sigma_{\text{el}}=0.5$). This is an unusual and unwanted situation in a Mössbauer experiment. In practice, therefore, a choice has to be made between a thin absorber which reduces the potential of the resonant effect or a thicker absorber which will result in a reduction in the spectral quality of the detected photo peak at 13.3-keV. The use of enriched ^{73}Ge absorbers are vital in this work.

Other difficulties arise from the extreme sharpness of the resonance. Narrow lines are only possible if the source and absorber nuclei are sited in environments as free as possible from all sources of inhomogeneous broadening. In the experiments reported, a fairly intense source of ^{73}As embedded in a single crystal of high purity Ge and a single crystal absorber prepared with enriched ^{73}Ge isotope were used. The velocity spectrometer employed a conventional electromechanical drive operating in the velocity range $\pm 88 \mu\text{m s}^{-1}$. This very small Doppler velocity, similar in magnitude to the velocity of the minute hand of a wrist



Energy spectrum of ^{73}As source together with decay scheme of $^{73}\text{As} \rightarrow ^{73}\text{Ge}$ (from Raghavan and Pfeiffer).

watch, is more than sufficient to scan the resonant line. This extremely small velocity range requires the apparatus to be isolated from building vibrations and the equipment was, therefore, set on a pneumatically suspended granite table.

Raghavan and Pfeiffer are optimistic, because of the importance of germanium technology and the extremely high purity and crystal perfection which can now be achieved by this technology, that the 13.3-keV transition of ^{73}Ge will become a powerful new tool in high resolution Mössbauer spectroscopy. The experimental problems, however, are quite formidable. Since α_T is so large, it would seem that a conversion electron detector which would detect the 1,100 internally converted electrons instead of, as in this paper, trying to detect the one resonant γ photon which escapes would reduce one of the experimental difficulties. Possibly one could use a $^{73}\text{Ge}(\text{Li})$ detector as both absorber and detector. There is no doubt, however, that ^{73}Ge Mössbauer spectroscopy does suggest many exciting possibilities to both the theoretician and experimentalist.

Reindeer overgraze in South Georgia

from our Plant Ecology Correspondent

THE introduction of mammalian herbivores by man has resulted in a number of ecological problems in many parts of the world. In the British Isles the introduction of the rabbit, probably in the eleventh or twelfth centuries AD, imposed a new constraint upon the development of many vegetation types. The effects of these animals in modifying the composition of grassland swards was ably demonstrated on the South Downs by Tansley and Adamson (*J. Ecol.*, **13**, 177; 1925) who first used enclosure as a technique for this purpose. Subsequently much work has been done on this subject, particularly since the myxomatosis epidemic of 1954 (see, for example, Thomas, *J. Ecol.*, **48**, 287; 1960; White, *ibid.*, **49**, 113; 1961).

The reindeer is another mammalian herbivore which has been transported to new areas by man, though not as widely as in the case of the rabbit. In 1909 eleven animals were released on the Antarctic island of South Georgia and further introduction continued until 1925 in other parts of the island. The populations have remained isolated from one another, but by 1958 the original Barff Peninsula herd had risen from 11 to 4,000 animals. Numbers have increased even further in recent years since hunting ceased in 1964. There is evidence that sheer pressure of numbers is forcing an extension of



Reindeer grazing *Deschampsia antarctica* sward now invaded by *Rostkovia magellanica* in the Whale Valley, South Georgia. Photograph taken in January 1972 by P. Stone (from Lindsay, *Bull. Br. Antarct. Surv.*, No. 35; 1973).

range despite unfavourable environmental conditions such as steep mountain slopes and glaciers with abundant crevasses.

Lindsay (*Bull. Br. Antarct. Surv.*, No. 35, 101; 1973) has reviewed the effects which high reindeer stock densities may be having on the vegetation of South Georgia. Unfortunately, past documentation of the flora has been largely non-quantitative and this makes it difficult to be sure that vegetation is indeed changing. To supplement this anecdotal evidence concerning the past flora of the island, Lindsay makes a comparison of vegetation in grazed areas with that in similar areas which as yet have no reindeer. This method of approach also has certain disadvantages, for one can never be sure that all other factors are equal in the two sites.

Despite these difficulties, Lindsay does present evidence which suggests that the intensive grazing of reindeer is severely modifying the composition of the vegetation. Some plants, such as *Acaena adscendens* are little changed in overall frequency, but are reduced to a creeping, rhizomatous growth with few leafy shoots. This species was recorded in 1890 as being "richly developed" in the area. In areas of very high stock density, such as the Barff Peninsula, *Acaena* is almost completely eradicated; evidently the constant removal of leafy growth can weaken the species over long periods. Some species such as the grass *Festuca erecta* and the rush *Rostkovia magellanica* are apparently unpalatable and hence have increased in abundance as a result of the reindeer's selective grazing. *Festuca*, however, does seem to be sensitive to heavy trampling.

The most serious effects of grazing and trampling are upon the lichens. Many species abundant in the ungrazed areas are now almost or completely absent from the grazed regions. The

two most abundant lichens affected are *Cladonia rangiferina* ("reindeer moss") and *C. furcata*. The former has vanished completely and the latter is reduced in cover abundance by a factor of ten as a result of the heavy grazing pressures. Experimental work on reindeer in the Arctic (for example, Pagau, *J. Range Mgmt.*, **23** (2), 95; 1970) has shown a similar demise of lichens under heavy grazing and trampling pressures. Such an effect is not of widespread importance in most Arctic areas because the density of reindeer is normally far lower than in South Georgia. For example, caribou in Newfoundland are estimated to have a density of one animal per 110 km²; densities in South Georgia may locally rise as high as one animal per 0.07 km².

The very high densities of reindeer attained in this new environment free from predation is adequate to explain the gross vegetational changes suggested by Lindsay's study. Two lessons should be learned from this work; in the first place the population levels of an introduced animal world should be strictly monitored and controlled and in the second place a more rigorous effort should be made to document the flora and fauna of areas in which such introductions are planned. Only in this way can the native biota be used as a clinical thermometer attesting the health or otherwise of a virgin community placed under an alien stress.

Questioning isostasy

from our Geomagnetism Correspondent

THE observations made by the members of Pierre Bouguer's expedition to Peru between 1735 and 1745 were to lead to the discovery of one of the most important principles in geology. Bou-

guer's principal aim was to measure an arc of meridian, a procedure requiring repeated determinations of the vertical—and he was fully prepared for the local errors arising from the horizontal attraction of the Andes on his plumb-line. But to his surprise he found that the gravitational pull exerted by the Andes was "much smaller than that to be expected from the mass represented by these mountains!" More than a century later, after Sir George Everest had found similar discrepancies in the vicinity of the Himalayas, Airy (*Phil. Trans. R. Soc.*, **145**, 101; 1855) was able to use his "hypothesis of crustal balance" to explain this curious lack of mountain power in terms of an underlying mass deficiency roughly equal to surface load represented by the mountains themselves. Thus was born the principle of isostasy, although the operative word was not coined until more than 30 years later by Dutton (*Bull. phil. Soc. Wash.*, **11**, 51; 1889).

Today, few people would dispute that at equilibrium there exists in the Earth a certain 'level of compensation' above which all columns of material having the same cross-sectional area have the same mass. Moreover, even the old conflict between Pratt's (*Phil. Trans. R. Soc.*, **145**, 53; 1855) suggestion that isostatic adjustment takes place by lateral density variation above a level of compensation lying at constant depth, and Airy's view that the material above the level of compensation comprises a lower density crust overlying a higher density substratum, has been settled to most people's satisfaction by acknowledging that either or both mechanisms may apply in certain circumstances. The basic principle of isostasy is now so ingrained in the fabric of geology that few earth scientists would question that the crust in a region thrown out of isostatic equilibrium by an 'external' force such as glaciation will return to such equilibrium once the force is removed.

But Artyushkov (*J. geophys. Res.*, **79**, 741; 1974) now questions precisely that, arguing that "the isostatic state is generally unstable and differs from the stable position of the crust, which actually exists in many regions". What apparently persuaded Artyushkov to carry out the analysis leading to this remarkable conclusion was the acknowledged fact that, for no immediately obvious reason, many young orogenic regions (the Eurasian Alpine geosyncline zone, for example) are associated with strong isostatic anomalies, even though gravimetric data show the Earth's crust as a whole to be generally close to isostatic equilibrium.

In developing this problem point, Artyushkov notes that in a region whose horizontal scale is several times greater than the lithospheric thickness

the crust will recover equilibrium quite rapidly following the removal of external stress. For a region several hundred kilometres across, for example, the characteristic recovery time following glacial retreat is only of the order of thousands of years. But in young orogenic regions the crust is hotter than normal and the lithosphere is thinner, implying an even more rapid and complete restoration of equilibrium. In other words, the period of crustal recovery in such regions is much less than the typical duration of tectonic activity (10^5 – 10^7 yr), suggesting that on a regional scale the crust should be close to its equilibrium position.

One way of reconciling this supposed equilibrium with the obvious lack of isostasy would be to invoke viscous forces acting on the lithosphere from the asthenosphere, but the creation of the necessary viscous stresses would require improbably high asthenospheric flow rates of greater than 10 m yr^{-1} . Instead, Artyushkov sets out to show that it is a mistake to assume that the equilibrium position of the crust is generally the same as the state of isostatic equilibrium; the two states are physically quite distinct and coincide only in special circumstances.

The core of Artyushkov's case is an extended mathematical treatment of an admittedly simplified model of the Earth's crust floating on the mantle. But it is sufficient to show that large non-hydrostatic stresses exist in the crust and lithosphere because of horizontal variations in crustal and lithospheric thickness and that these stresses are non-uniformly distributed throughout the depth. One consequence is that the crustal/lithospheric layer has two characteristic relaxation times, the first (T_1) being associated with the vertical adjustment of the layer and the second (T_2) with the horizontal spreading of inhomogeneities in the layer thickness. T_1 , which is much smaller than T_2 , is the time associated with the reaching of the 'equilibrium position' (that is, the mechanical equilibrium position) and this state only coincides with isostasy in the special case of a region where the crustal and lithospheric thicknesses are constant; conversely, unless the thicknesses are constant the state of isostatic equilibrium is mechanically unstable.

Because data on the vertical distribution of the stresses in the lithosphere are sparse, it is not possible to compare gravity anomalies predicted by the Artyushkov model with observed anomalies in any great detail, although there is general agreement between the two. Thus the model predicts very low gravity anomalies for platform lowlands located far from mountain regions, negative anomalies of a few

tens of mgal over ocean basins a few thousand metres in depth, and positive anomalies of a few tens of mgal over young orogenic regions—to take just a few examples. Practical demonstrations apart, however, the more important general point is that Artyushkov's analysis offers an explanation, in principle, of certain isostatic anomalies whose existence geologists and geophysicists have very often tended to gloss over. By the same token, it also seems to hit at one of earth science's most cherished principles, suggesting that even the most basic and time-honoured assumptions may benefit from critical re-examination.

Sticks and carrots

from our Materials Science Correspondent

AT a conference on the conservation of materials, sponsored by the Institution of Chemical Engineers and the British National Committee on Materials, on March 26–27 at Harwell, the emphasis was partly on primary metal supplies and the associated topic of substitution, with a low in the direction of plastics. Much of the conference, however, was targeted on the problems, both technical and political, associated with recycling. The political interest in this field was signalled by the opening address delivered by the Chief Scientific Adviser to the Cabinet, Sir Alan Cottrell, and of the Liberal peer, Lord Avebury.

What was for one observer the most interesting issue discussed at the conference was raised by P. F. Chapman (Open University)—a most cogent and persuasive speaker. His primary concern is with the energy content of primary metals implied by the energy input of the extraction and purification procedures. When this aspect is pushed into the forefront, then the systematic recycling of 'energy intensive' metals becomes particularly attractive. The problem is, how are the public and manufacturers to be persuaded to take the necessary steps—domestic conscientiousness and apposite engineering design—to increase recycling substantially?

Chapman quoted the parable of the common. A piece of common land can support twenty-five cows, and five smallholders each have the right to graze five cows there. All is well until one of them adds a sixth cow, to increase his income a little. All twenty-six cows go a little short of grass, including the rogue smallholder's sixth cow, but overall he gains; most of the loss, without any compensating gain, falls on the other four smallholders. In turn, they add cows, until in the end all are worse

off, and the last state is worse than the first for all. This might be a parable for the evils of inflation, but in the present context the point is that the incentive for self restraint is merely collective, whereas each individual in isolation has a financial motive for selfishness.

A case in point is the design of beer cans. Many of these are made of steel, with a top made of aluminium for its easier tearing qualities. Such cans are almost useless for recycling, since aluminium is an unacceptable contaminant of scrap steel. The manufacturer has an incentive to design a can in such a way that the consumer is most likely to buy it (I take it as axiomatic that most canned beer is of indistinguishable and mediocre quality!). The scrap merchant or the British Steel Corporation gains from a prevalence of aluminium-free cans, whereas the beer drinker and the can maker do not, and may even suffer inconvenience.

This last point, the fact that the financial benefit of improvements in the recycling technique does not go to the people who need to modify their behaviour to bring the benefits about, was also made forcefully by J. B. J. Williamson (Guest, Keen and Nettlefolds Ltd). He worded it differently: in the course of materials recycling, ownership of raw material changes from manufacturing industry to the user, until it becomes waste and belongs to everybody and nobody. This is why recycling in the manufacturer's plan ('prompt recyc-

ling') is so much more effectual than 'post-consumer recycling'. A recycling industry is defenceless against the vagaries of the public, and is so vulnerable to a fierce price-war with primary producers that it would have to concentrate totally and would have no scope for diversification—hence would be unattractive for investment. This difficulty is enhanced by the fact that technological improvements by users reduce demand for metals in short supply; thus a new, effective method for joining aluminium cable ends reduced demand for copper cables.

Yet an autonomous recycling industry is bound to come. Williamson was persuasive in arguing that the economic forces of the market will not favour recycling; but recycling will become much more attractive if (1) foreign primary sources become less reliable (he had disruption in mind rather than nationalisation) and (2) governments convert environmental priorities into fiscal incentives and threats.

This will not be easy—Chapman suggested that the building of aluminium smelters in Britain with government backing and incentives led to a reduction of aluminium recycling rather than a reduction in primary imports—but it is essential. Plainly manufacturers and consumers alike must either be cajoled with allowances and other financial incentives (for instance, to use all-steel or all-aluminium cans, and then to collect them after use) or they must be threatened with penalties if they do not

behave. The trouble with the stick is that civil servants have to think up a complete programme of legislation and the police have to enforce it. Yet such a programme can only be based on present technology: as Marx commented, the problem is not to understand the world but to change it, and the advantage of using the carrot instead of the stick—incentives rather than threats—is that industry would have a motive for developing new recycling processes and new manufacturing designs congenial to recyclers. There must be a mixture—but let there be incentives for carrot growers.

Sir Alan Cottrell remarked that the British Government will usually act if they have clear, unambiguous information. It is to be hoped that the Civil Service will provide that information and that legislation will follow.

Dynamics of biomembranes

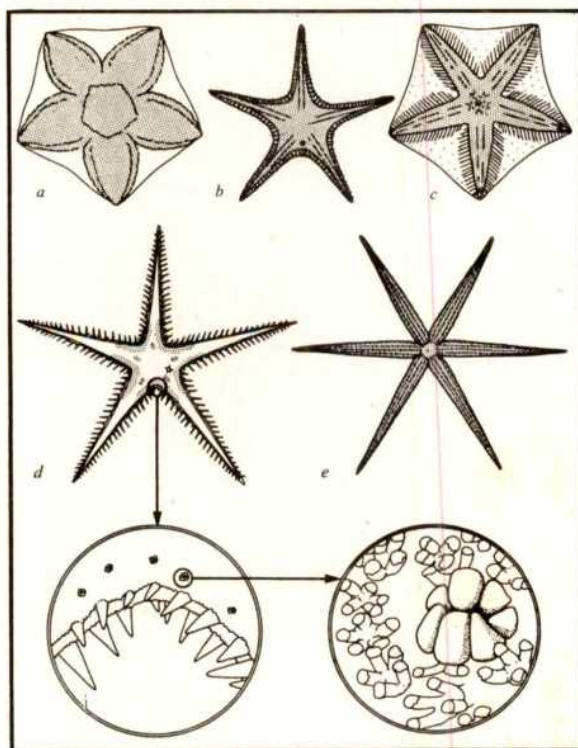
from a Correspondent

AT an international conference in Titisee on March 21-23 on the dynamics of biomembranes, the proceedings began with a reminder by H. P. Zingsheim (Max-Planck Institute, Göttingen) of the difficulties of attempting to draw conclusions on the molecular structure of membranes from either fixed and stained sections or from freeze fractured preparations. Chemical studies by M. S. Bretscher (University of Cambridge) and investigations with purified phospholipases by R. F. A. Zwaal (University of Bern) which have revealed an asymmetric distribution of phospholipids in red cell membranes were discussed. There is, however, no evidence for similar asymmetry in other cell types: the biosynthetic origin of the asymmetry is also obscure. In connection with lipid phase transitions, D. Chapman (University of Sheffield) reported that transition temperatures can be altered by drugs, especially antidepressants, and H. Träuble (Max-Planck Institute, Göttingen) indicated that lung alveoli may utilise the phase transition properties of surfactant phospholipids during expansion and contraction.

Interesting points were made by E. H. Eylar (New Jersey) on two autoimmune diseases induced by injection of membrane proteins that may normally not be exposed *in vivo* to the immune system, namely, the A1 basic protein of the myelin of the central nervous system and sperm proteins: the sperm proteins have a possible immunological application in the control of fertility. Complex formation of the A1 basic protein with lipids in surface monolayers was dis-

Luminescent echinoderms

Like many other marine animals, some echinoderms are bioluminescent. Herring (*J. Zool.*, 172, 401-418; 1974) has found luminescence in two species of ophiuroids, six asteroids, nine holothuroids and two crinoids. This figure shows the main luminescent areas (stippled) of five asteroids: a, *Cryptasterias personatus*; b, *Plutonaster notatus*; c, *Hymenaster* sp.; d, *Pectinaster forcipulatus*; and e, *Hydrasterias ophidion*. The dorsal surface of *P. forcipulatus* bears fasciculate pedicellariae which do not occur elsewhere on the body but it is not known if they are involved in the luminescent response.



cussed by A. A. Demel (University of Utrecht) who reported that the amino terminal region of the protein seems to interact preferentially with lipids as this region is resistant to trypsin added to the subphase.

On the second day J. A. Lucy (Royal Free Hospital School of Medicine, London) drew attention to the possible role of increased lipid fluidity in membrane fusion. C. A. Pasternak (University of Oxford) then described experiments on membrane leakiness (not lysis) which may be an initial stage in virus-induced cell fusion; he also ventured the opinion that enzymes are not involved in this action of viruses. K. Simons (University of Helsinki) presented findings on the membrane glycoproteins of Semliki forest virus which resemble glycophorin in projecting right through the membrane. Considerable discussion was aroused by his intriguing proposal that release of virus by budding through the plasma membrane may involve recognition and interaction of nucleocapsid proteins with the cytoplasmically-exposed segments of these glycoproteins in the membranes of infected cells.

S. C. Kinsky (Washington University, St Louis) reported important studies on liposomes which he had rendered immunogenic by incorporating DNP-coupled, N-substituted derivatives of phosphatidylethanolamine. Appropriately substituted phospholipids thus seem potentially valuable in immunological studies on haptens: they may also be useful in the radioimmunoassay of drugs. Another investigation on liposomes was described by B. E. Ryman (Charing Cross Hospital Medical School, London) who has encapsulated enzymes within liposomes (of potential use in inborn errors of metabolism), and who is applying encapsulation to drugs, for example, 5-fluorouracil, as this may permit the tissue specific application of relatively small doses of drugs.

Lysolecithin was a recurring topic, and there were discussions of whether or not small quantities might be involved in virus-induced cell fusion, of its acylation with unsaturated fatty acids in thymus cells stimulated by concanavalin A, its possible role in cellular junctions and its facilitation of concanavalin A-induced agglutination in chicken erythrocytes during which H. Fischer and his colleagues (Max-Planck Institute, Freiburg) made several contributions.

A lively exchange centred around the relevance of Mitchell's hypothesis in the transport of galactosides, amino acids and other molecules across membrane vesicles prepared from *Escherichia coli* that is coupled to the oxidation of D-lactate (H. R. Kaback, Roche, New Jersey; W. R. Konings, Haren, Nether-

lands). Finally the mode of action of antibiotics on membranes, which seems still to be a very open question, was pursued by B. Deuticke (Technical High School, Aachen) who concluded that the presence of proteins scarcely modifies the action of these drugs on the lipid components of membranes, and by B. de Kruffy (University of Utrecht) who discussed the formation of some interesting, albeit speculative, molecular complexes of polyenes with cholesterol in relation to their biological activity.

The meeting was sponsored by Dr Karl C. Thomae, Biberach/Riss, subsidiary to C. H. Boehringer, Ingelheim and was organised by L. L. van Deenen (University of Utrecht) with H. Schroeder (Thomae).

Is the bass on the decline?

from our
Marine Vertebrate Correspondent

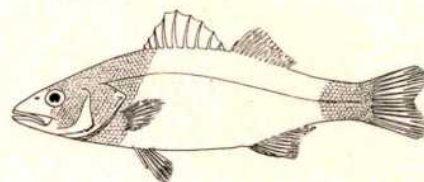
THE bass (*Dicentrarchus labrax*) is a well known species to the sea angler; it is highly rated as a fighting fish, it grows to a fair weight, and has a relatively palatable flesh. In British waters it is widely distributed, but common only in the south and south-west, although it occurs as far north as Scotland where especially in the north-east it is rare. Until recently, however, its biology was virtually unknown; there were no data on its ageing and growth, little was known about its food, and the only knowledge of its spawning habits and early life was derived from elementary observations. The value of the bass to anglers first prompted research by staff of the Inland Fisheries Trust, Dublin who contributed to knowledge of the bass's biology in Irish waters (Kennedy and Fitzmaurice, *J. mar. biol. Ass. U.K.*, **52**, 557-597; 1972). Knowledge of this fish has now been extended in a report by Holden and Williams on dynamics of bass in English waters (*J. mar. biol. Ass. U.K.*, **54**, 91-107; 1974).

Holden and Williams's study was initiated by the concern expressed by angling bodies at the apparent decline in bass populations in English waters. A programme of tagging was begun with the aid of members of the National Federation of Sea Anglers, in which anglers tagged 954 bass in 1970 and 1971 mainly on the coast of Devon. Of these fish, 59 were recaptured, most (45) within 16 km of their release point and the majority (44) within the year of tagging. The exceptions, however, show that there were long-range movements, some of them over an extended period, the longest being 1,066 days at liberty. Most of the fish represented by returns had evidently made coastwise migrations, although one fish tagged on

the South Devon coast was captured after 222 days in mid-Channel to the west of the Channel Isles.

On the basis of these tagging returns the authors suggest that the life history of bass in English waters falls into two phases. The first is the nursery stage when young fish of about 10 cm length enter estuaries in which they stay for 1 or 2 years, leaving usually as 3-year-old fish. The second phase is one of greater mobility when, as adult fish, they migrate coastwise and even offshore, possibly seasonally.

This synthesis of the first years agrees with the results of Kennedy and Fitzmaurice who also found young bass to live in estuaries, creeks and lagoons, but concluded that mature bass do not make extensive migrations. The different conclusions of Holden and Williams may be due to the fact that the Irish tagging was confined to the southern coasts of Ireland where bass are abundant and present all year round. A substantial part of the tagging effort of Holden and Williams was expended in similar areas of south-west England, however, and the data in confirmation of their theory amount to only 12 returned tags, of which only 6 were of distant migrations. In support of this rather slender data they cite communicated information from another source of three bass tagged off Anglesey which were recovered in Cornwall and Devon, but of this critical evidence no further information is advanced.



Dicentrarchus labrax.

The apparent contradiction between the conclusions of the two groups is important. If Kennedy and Fitzmaurice are correct in assuming that the bass populations are mainly sedentary, then heavy fishing could quickly lead to severe local depletion of the stock. Conversely, if Holden and Williams's conclusions are accepted the stock of bass is at least national, and heavier exploitation can be permitted locally without undue damage. It is unfortunate that in this critical area there is conflict of opinion and little data from which to draw conclusions.

With regard to the alleged decline in bass fishing, both pairs of authors consider the cause to be the failure of successive year-classes caused ultimately by climatic changes. Holden and Williams even venture to predict that if present climatic trends continue, bass could possibly become a rare species even in southern British waters.

Fortification of foods with amino acids

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In this article Dr Altschul discusses how the addition of specific amino acids can solve some nutritional problems, chiefly by reducing the pressure on protein produced from the land.

FORTIFICATION (or supplementation) of a food with amino acids is a process whereby the limiting deficiency of amino acids in a diet is eliminated or reduced by adding small amounts of one or a small number of amino acids. As such it becomes a practical tool for selectively improving the protein component of a diet without requiring the addition of other amino acids or food components already present in adequate amounts. Usually, the deficiencies of one protein source are balanced, in part, by mixing with one or more other protein sources which contain an excess of the amino acid deficient in the first: corn plus beans, or a cheese and bread sandwich, for example. The result is the creation of a new mixture of better protein value than the first. The use of specific amino acids allows adjustment of the amino acid composition in the protein to the exactly desired balance, that is in balance with the second limiting amino acid. Such adjustment introduces another option for increasing protein supply at low cost.

Supplementation with amino acids is normal practice in formulating animal feeds¹. For example, corn and soybeans are the major energy and protein ingredients in nonruminant feedstuffs in the United States. When soy represents a substantial portion of the protein in the mixture with corn, then methionine is limiting; addition of DL-methionine improves the protein quality of that mixture. When soy provides a small proportion of the protein in the diet, the mixed feed does not provide enough lysine to balance the deficiency of lysine in the corn. In such circumstances addition of lysine is required for optimum nutrition. This can be obtained from fish meal or by addition of the amino acid itself.

Addition of amino acids also can be part of a system of improving the quality of human diets. Addition of methionine makes certain legume foods better sources of protein. It has been suggested that the addition of less than 1% of methionine to manioc flour would improve the value of cassava bean diets in Brazil by improving the protein quality of the beans². Bread supplemented with lysine is a far superior source of protein than ordinary bread. Fortification may be a means of reducing the cost and improving the delivery of protein in institutional meals and could also improve the special delivery systems organised for infant and maternal feeding or for feeding of the aged.

Hegsted³ estimated that white flour containing 3.2% utilisable protein will have its protein impact increased to 5.3% on addition of 0.2% L-lysine·HCl. On this basis it can be estimated that the one million tons of wheat consumed in Tunisia annually would gain the equivalent of 20,000 tons of utilisable protein by addition of lysine. Relief shipments of grain to avoid famine can be made more complete sources of nutrients, particularly protein, by fortification either at the point of shipment or at destination.

Nutrition is not merely calorie or protein nutrition; it is meeting the total nutrient requirements of the body. Thus, mechanisms which allow addition of amino acids to

single foods or diets can also be the means of adding other micronutrients such as vitamins and minerals.

The literature on amino acid fortification has been reviewed extensively⁴⁻⁷. Eight essential amino acids—leucine, isoleucine, lysine, methionine, valine, threonine, phenylalanine and tryptophan—are required by human adults; histidine is required in addition by infants. The highest requirement for the infants is leucine and the lowest is tryptophan. Adults require less protein and less of the essential amino acids—19% as essential compared with 40% for the infant—and the spread in requirements among the essential amino acids is also less than for the infants. Radical departure from the optimum pattern can cause amino acid imbalance with many consequences including reduced growth^{8,9}. This can result from excessive addition of a single amino acid to a diet or from complete reliance on single sources of protein such as corn¹⁰. Thus, any supplementation with amino acids must be controlled to avoid such imbalance.

Amino acids rarely occur free in nature; they are supplied as proteins in food. Essential amino acids and the nitrogen required for growth and maintenance of body tissue nitrogen are thus supplied by the food protein. Amino acids may also be supplied as protein hydrolysates or as mixtures of free amino acids, the latter either orally or parenterally. The metabolism of amino acids presented as proteins differs somewhat from that of free amino acids, particularly in the treatment of temporary excesses, but all the evidence supports the idea that for practical purposes amino acids in proteins and free amino acids can be considered as interchangeable¹¹.

Certainly there is every evidence that a food protein supplemented properly with amino acids will have the expected improved impact. Many generations of rats have been raised on totally synthetic diets in which proteins were completely replaced with specific amino acids and other sources of nitrogen¹². Humans have been kept in nitrogen balance by completely synthetic diets consisting entirely of the essential amino acids and other nitrogen sources¹³. Infants grow normally on wheat protein plus lysine as the sole source of protein¹⁴. However it is measured, the fact emerges that plant sources of protein properly supplemented with amino acids are adequate to support growth in animals and man.

Amino acid supplementation in practice

By far the greatest utilisation of pure essential amino acids has been in animal feeds; the most used has been methionine. The United States consumes about 22,000 tons of DL-methionine annually; world capacity is 92,000 tons¹⁵. Prices for DL-methionine are in the range of \$1.20 to \$1.60 per pound. Recently, as the price of soy has increased, formulas are being reconsidered to use the least amount of soy protein necessary to complement the amino acids of corn. For many feeding purposes the protein content of the diet can be relatively low; the soy then is required primarily to furnish tryptophan; the deficit of lysine can be overcome by providing additional L-lysine. Consumption of lysine in the United States is at the level of 6,000 tons annually at a price of \$1.00 to \$1.60 per pound.

TABLE 1 Estimates of cost of fortifying cereals with amino acids

Case 1: Rice²⁹

Ingredients	Cost Assumptions	Cost Estimate
Rice (milled)	\$200 per 1,000 kg	
L-Lysine HCl (0.15%)	\$1.80 per kg	\$13.60 per 1,000 kg
		or
L-Threonine (0.05%)	\$19.40 per kg	6.8% of cost of rice
Fortification granules and cost of addition to rice	\$1.20 per 1,000 kg	

Case 2: Wheat*

Wheat	\$200 per ton	
L-Lysine HCl (0.23%)	\$10 per ton of wheat	
Vitamin A (250 million IU g ⁻¹) (0.033 g kg ⁻¹)		\$12 per ton.†
		or
Niacin (0.019 g kg ⁻¹)	\$2 per ton of wheat	6% of cost of wheat
Riboflavin (0.0025 g kg ⁻¹)		
Thiamin (0.0016 g kg ⁻¹)		
Iron (0.055 g kg ⁻¹)		

* P. R. Crowley, personal communication.

† Cost of ingredients includes cost of preparing and adding a premix.

Methionine has been added at 0.5% of soy protein in infant formulas; such formulas have been judged equivalent in protein value to the proteins of meat, eggs, and milk¹⁴. Interest in applying lysine fortification to human foods is relatively recent and coincided with the development of methods for the production of L-lysine at practical costs. It was recognised early that lysine supplementation offered an unprecedented opportunity to improve protein nutrition, particularly for those who could not afford adequate protein and who depended heavily on cereals for most of their calories and proteins¹⁷⁻¹⁹.

The applications are few however. Some cold breakfast cereals and breads are fortified with lysine in the United States. There are instances of rice and noodle products fortified with lysine in Japan. Certain Incaparina formulas are fortified with lysine²⁰. Interest in amino acid fortification of human foods is certainly on the increase but at the time of writing, the utilisation of this option is at too low a level to make a significant contribution to human protein supply.

The economics of amino acid fortification of human foods are complex. The population is heterogeneous compared with the homogeneous animal populations; different human age groups differ in their requirements of protein and lysine. Moreover, not all diets are always deficient in protein. The poor, and particularly the vulnerable among them, are most likely to be deficient in protein. Amino acids improve protein quality, they add no calories to the diet; thus, it is difficult to compare in a general way costs and benefits of this approach to nutrition compared with other options. The comparison must be on specific approaches to well-defined groups.

Issues surrounding amino acid fortification

The main issue is how to use amino acid fortification to advantage to provide more protein at a lesser cost to people who do not get sufficient. Since this will involve a government decision to allocate scarce resources or to modify regulations involving addition of amino acids to the food supply, the ease and rapidity with which such a positive decision is made depends in part on the nature of the consensus among scientists who advise government. The following are some of the issues being discussed within various countries and in United Nations' agencies regarding amino acid supplementation of foods.

Calories or protein This involves amino acids only peripherally. The question is whether the most important deficiency in the diets of poor nations is protein or calories.

Amino acid addition represents the extreme of adding protein under circumstances where no additional calories are provided.

Concern for the protein aspect of malnutrition was intensified by the discovery of the aetiology of kwashiorkor, recognition of its wide distribution in underdeveloped countries, and the realisation that clinical protein-calorie malnutrition is only a small aspect of a more general syndrome involving specific protein deficiency²¹. More recently, important members of the nutrition community have insisted that the major problem in poor countries is lack of calories, that the protein quality is adequate, and that what is needed is more of the same kind of food²². Latest revisions of standards of protein requirements for normal healthy humans are lower than before^{23,24}. Although there are many uncertainties, such as the increased needs of the sick and the relationship between minimal and optimal protein nutrition, the tendency is to translate such nutritional judgements into recommendations for public policy.

The meaning of 'more of the same kind of food' needs more inquiry. The proven existence, or lack, of protein malnutrition is just one part of the issue. The major problem is the interdependence of calorie and protein supply: there is a protein-calorie tradeoff. This occurs because calories are cheaper than protein. Poverty forces a reduction in the amount of protein food consumed (legumes or animal products) in favour of the cheaper foods (cereals, root starches and sugar).

Stress for more calories challenges agricultural capacity to maintain quantity and quality of protein supply. Increased population demands an increased food supply merely to maintain nutritional conditions at the existing level. But there is no unlimited ability to expand food production. The great increase in grain production—the "Green Revolution"—which has unquestionably had a great impact on food availability has come about because high yield varieties of wheat and rice were planted. These required more fertiliser and energy input and more irrigation. More acreage was put into grain production at the expense, in some instances, of that devoted to legume production.

In India, for example in the sixties, *per capita* calorie availability fluctuated around 2,050 kilocalories: first dropping to 1,950, rising to 2,200, and then returning to 1973 estimates of 2,050. Protein availability remained about the same in that decade at about 53–55 g *per capita* per day. This is a great achievement considering the population increase during this period, but percentage of protein from pulses (legumes) decreased from 23% in 1959–61 to 15% in 1973. Without a compensatory increase in animal protein, this trend represents a serious threat to protein balance, particularly to the lysine content of the diet²⁵. An FAO report of projections of percentage of calories as protein in South Asia lists this value as decreasing from the present 10.2% to 9.8% in 1980 (ref. 26). As the percentage of protein calories is reduced, more reliance is placed on cereal protein and less on concentrated protein sources such as legumes and animal proteins, which also have an excess of lysine. Therefore, not only is the percentage of protein as part of the calories reduced but also the quality of the protein. The reduction is far more serious than the numbers would indicate.

Added to this problem is the increasing demand for protein by nations whose income *per capita* is increasing. Those that have had high incomes for some time have enjoyed a high protein intake gained at the expense of huge excesses of cereals to feed the animal population. In the United States approximately one ton of cereal is available *per capita* but only about 150 pounds are eaten each year by humans directly; the rest is fed to the animals. The grain equivalent calories required *per capita* per day in the developed countries can be estimated as 8,000 kilocalories

compared with 3,000 for the underdeveloped countries^{27,28}. As their income increases, other countries are trying to catch up, as evidenced by the increased sales of cereal grain and protein concentrate to Russia and Japan, as well as Western Europe. Thus, there is the situation where, almost explosively, the world has been forced to recognise a worldwide shortage of protein or the ingredients with which to produce it. The United States had a temporary embargo on the export of protein-rich commodities and feedstuffs in the summer of 1973.

In the face of such developments it is inconceivable that any country or any group can say that there is no protein problem. Certainly, planning on that basis courts disaster.

Natural or synthetic sources of amino acids There is a preference to obtain the deficient amino acids by breeding them into the plant rather than by supplementation. This idea has attractive features. It departs least from the norm: the corn, wheat or rice is almost the same, but has more protein and lysine. If this could be achieved finally without any reduction in yield for the particular crop, then this is indeed the best idea.

The cereal most advanced in this direction is corn. High lysine corn has made considerable progress²⁹. Nevertheless, the new corn has not been planted in a practical sense any more than amino acid fortification has been instituted, because in most instances the yield is less, there are some problems of acceptance of conventional food products derived from these varieties, and there is need for social organization to ensure that the correct seeds are planted and sold. Whether or not it will ultimately be possible to develop the improved corn so that it will yield and maintain increase in yield as well as the ordinary corn remains an open question. At present yield per acre is all-important. A more likely outcome could be a combination of breeding for improved quality with fortification to complete the process.

Unfamiliarity with the concept Supplementation with amino acids or vitamins and minerals is an unfamiliar concept in most places. To increase familiarity and provide a means for seeing what could happen in reality, three field trials have been organised by the Agency for International Development (US) in cooperation with the United States Department of Agriculture, several governments and their scientists, academic institutions, and some industrial firms providing ingredients and technology^{30,31}. These involve addition of lysine and vitamins to wheat flour in Tunisia; fortification of rice with vitamins, lysine, and threonine in Thailand by mixing synthetic granules with natural rice in the village mill³²; and fortification of corn at the village mill with small quantities of soy flour plus lysine in Guatemala.

In all instances baseline data are being obtained on the populations, particularly the children, and information is being collected on the cost of fortification, its effect on the amino acid composition of the food supply, and on whatever changes can be determined in health status that might be attributed to improvement in the protein value of the foods. It is realised, of course, that measurement of changes in an entire population and the determination of the important cause and effect relationships is difficult, if not impossible. Certainly, that fortification with amino acid improves the protein value of a cereal is beyond doubt. The issue is the degree to which the effect of nutrition intervention alone on public health can be determined.

Cost Since the object of amino acid fortification is to reduce the cost of protein for those who can least afford it, government subsidies are involved, so the cost of amino acids and lysine, in particular, is critical. Sample calculations of the cost of fortification of wheat and rice are given in Table 1.

Slessor³³, in his discussion of energy subsidy in food production, considered the case of fortification of 'Food for Peace' shipments of wheat from the United States in 1966. To have fortified 30×10^9 pounds of wheat with 32×16^9

pounds of lysine would have required an energy subsidy of 390×10^9 kilocalories, and this would have provided a nutritionally feasible diet for one hundred million people. He also said that the same energy subsidy expended in the importing country on marginal grassland could produce 38 pints of milk per person. Extrapolation of the calculation derived earlier³ to this case shows that the fortification route provides a gain of 6.3 pounds of useable protein *per capita*; the same amount of subsidy would yield 1.2 pounds of milk protein *per capita*.

Amino acids are produced either by fermentation or by direct synthesis³⁴⁻³⁶. Lower levels of production favour fermentation; at greater concentrations, economies of scale favour synthesis of lysine. Thus, the generator for cost reduction will be the volume of amino acids utilised in animal feeding. Mitsuda and Yasumoto⁷ and Durrenmatt³⁷ showed that the trend of production cost of other synthetic nutrients would suggest a further lowering in cost of lysine. It has been estimated that in large scale production, lysine can be produced for about \$0.50 a pound or less.

Present world lysine production capacity is estimated as 16,000 metric tons annually¹⁵. It is predicted to reach a level of 60,000-70,000 tons by 1980, primarily because of increased demand as a feed supplement. In some areas demand far outpaces supply; in France the price of L-lysine quadrupled to \$9.30 per kilogram in 1973.

No substitute for aesthetics The supplementation of cereals or foods is a completely nutritional manoeuvre. It is not visible; and that is its advantage as it requires no change in eating habits, but by the same token, it is difficult for a government to claim credit for such expenditure without obvious immediately visible beneficial results.

This is not a manoeuvre equivalent to adding meat to the diet, or legumes. Calculations can be made of the meat or milk equivalent in protein added to a nation by fortifying a given cereal grain supply with an amino acid. From a strictly protein point of view, these might be correct. They are not, however, entirely correct because the meat adds calories and other nutrients as well as improving the protein quality of the cereals. More importantly, meat adds variety and a psychological stature to a diet which no amount of fortification can induce. There is no doubt that the use of animal protein as a means of improving cereal protein is the preferred way of improving diets when income is increased.

It is tempting to conclude that where the quest for aesthetic satisfaction in foods predominates, no amount of fortification, be it with amino acids, vitamins, minerals, or protein concentrates, can gain the political support that nutritional considerations warrant. It would, therefore, be expected that fortification will make the greatest initial progress in countries and socioeconomic groups where there has been a modicum of aesthetic satisfaction in foods and where aesthetics are no longer the burning issue. Yet, the appreciation of nutrition as apart from eating as an essential component of public health measures is probably far greater than assumed. It is possible, therefore, that in some areas a purely nutritional measure can gain political support on its own public health merits.

Projections

The two basic constraints on increased food production are land and energy, and the two are interrelated. Where shortages of the two exist, in the short term the picture is grim. Increases in yield were made possible by engineering food plants to withstand and derive benefit from increased energy inputs. An improvement in the present land constraints requires that plants be engineered for higher photosynthetic yield at constant energy input. The development of new sources of energy which will change the nature of the energy constraints is the most likely long term effect. The practice

of using more nonagricultural inputs to maximise food production will continue. Just as this trend will be reflected in optimum use of water, fertiliser, insecticides and fungicides, so will it by increased usage of nonagricultural food nutrients to enhance the quality of the products of the farm.

It has been estimated that 10,000–20,000 tons of L-lysine will, by 1980, be added to cereals intended for human food¹⁵. The rapid proliferation of animal analogues from soy and other vegetable protein sources will increase the use of methionine in foods based on the meat and milk model³⁸.

Fortification with amino acids alone obviously does not solve all problems of nutrition. It does not provide calories. It does not provide aesthetic satisfaction in foods except where it contributes to the development of meat and milk analogues. It is no substitute for better distribution of wealth either within a country or worldwide. But it is a way of reducing pressure for protein from land. It could be considered by some as a temporary measure to bolster protein supplies until more attractive options become available. But, fortification with amino acids will probably become an essential and regular adjunct of human food systems. In this way, technology will contribute more flexibility to counteract the pressures of greater populations and higher expectations. Nevertheless, the pressure of population constitutes a burden that no amount of technology can expect to overcome indefinitely without a genuine lowering in the quality of life for almost everyone.

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Nutritional lessons from the Ethiopian drought

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The drought in Ethiopia and the subsequent international reaction has drawn attention again to the urgent need to consider methods of early assessment of and appropriate responses to such situations. Assessment requires the collection of reliable data; responses should pay attention to established nutritional principles and take account both of the situation as it exists and of the normal dietary patterns.

THE population of Ethiopia is 25.2 million¹, of whom at least 90% live by subsistence farming. Ecologically there are a number of different zones, distinguished particularly by altitude and by local variations in climate. In the northern area, where relief efforts were concentrated in 1973 (see Fig. 1), there are broadly three zones: highland (above about 2,500 m), lowland and desert. The farming pattern is mixed: the main food supply is from crops of cereals and legumes in the highlands and lowlands, with increasing amounts of livestock

as the altitude decreases. The two food sources, crops and animals, are interdependent in that oxen are essential for cultivation. The desert people are largely nomadic herders, dependent on cattle and trading with some agriculture, and form a distinct group: they have had limited contact with the mainstream of Ethiopian life, and regard themselves as a separate community.

The land is intensively cultivated and there has been progressive deforestation and soil erosion. Even under normal climatic conditions little excess of grain is produced, particularly in lowland areas, and usually there is a hungry period of one or two months when the previous year's stocks of grain have been exhausted and the next harvest is not yet ready. Excess of livestock creates a little wealth: during times of shortage, animals are sold for meat and hides to buy grain when necessary, albeit at high prices.

The community was particularly vulnerable to climatic change. Between 1966 and 1973 the rainfall was erratic, resulting in poor harvests and inadequate grazing for livestock; local food shortages occurred periodically during this time, leading to population movements and government relief.

There are two annual harvests in most parts of Ethiopia, associated with the main rains (occurring between July and September) and the short rains (usually falling at some time between February and April). The harvest from the main rains, gathered in October to December, provides much of the food supply for the following year, supplemented by crops sown during the short rains; this latter harvest is also of critical importance in providing seed for the main sowing later in the year.

In early 1973 the short rains failed completely in many areas, particularly in Wollo and Tigray provinces. The harvest in late 1972 had been generally poor, and with the subsequent failure of the 1973 early harvest food stocks began to run out, particularly in the lowland areas, from April onwards. In the desert the drought probably had an even more disastrous effect in that little or no grazing remained by the early part of the year. Serious disruptions of the normal market equilibrium occurred. The cost of grain bought by merchants and sold in the large towns rose steeply, while the price obtained for cattle, sold in order to buy grain, fell from Eth\$ 120–150 to about Eth\$ 10. The abattoirs were unable to cope with the influx, so that many cattle, already in very poor condition, died before they could be slaughtered.

In the period from April to August increasing numbers of people, their food supplies in their villages exhausted and their cattle dying from starvation and disease, made their way to the roads and on to the towns. Little information was available as to the conditions in the countryside away from the roads, although it was assumed that many must have died. It was therefore clear at this stage that immediate action was required to prevent widespread malnutrition.

Faced with large numbers of refugees from the drought-affected areas crowded into the towns, sleeping in the open, the local authorities erected temporary shelters. By September, more than 60,000 people were accommodated in 12 shelters in Wollo alone. Food supplies had not yet been established and the construction of latrines was begun through force of circumstance after the shelters were already occupied, resulting in a high incidence of enteric infections. At the beginning of the rains each year there are anyway sporadic epidemics of enteric infections; these and other diseases common in crowded conditions added to the public health problems in the camps.

In April 1973 an Ethiopian government report indicated the seriousness of the drought and food shortage in Wollo, Tigray and northern Shoa provinces. Distribution of grain was begun by the authorities and outside food aid sought; later seed for the next harvest was also distributed. A relief fund and relief committees were established, and considerable

sums of money raised within Ethiopia. It was estimated from official records that nearly 2 million people were affected, and the grain requirement was calculated as of the order of 50,000 tonnes a month. Nothing like this amount was available and the problems of distribution were enormous. Mission and other organisations were receiving reports of drought and starvation, and were also active in providing supplies and help. The drought in the Sahel area was receiving much attention, and in July a meeting in London discussed "Drought in Africa"², including Ethiopia. By September there was widespread concern over the situation in Ethiopia, and further information was forthcoming, notably from UNICEF. At this time national and international agencies became involved, and aid began to arrive in quantity in October. By the beginning of 1974 teams from Britain, Germany, Holland, Switzerland, Australia, the United States and elsewhere were represented, most arriving several months too late to help the Ethiopians with the main problems, which were at their peak before the 1973 harvest. Relief was required both in the camps and in the remote rural areas but until recently was restricted almost entirely to the camps. Here the need was most obvious but their total population after October was not more than 15,000, less than 1% of the affected population.

The areas of interest concerning nutritional relief are

- defining the situation with respect to the nutritional status of the population, and
- deciding on the appropriate response in terms of food aid.

Assessing the situation

Most assessments of the situation were obtained from official records in centres of population, and from subjective individual reports. The frequently-quoted figure of 50,000–100,000 deaths (see, for example, ref. 3) was obtained from the records of a few towns, scaled up by an estimated factor to represent the total affected population. Rural conditions could only be assessed from nutritional surveys.

The available information indicates that by October 1973 stock losses of at least 80% had occurred in many areas, that 50–70% of the land was planted, and that the harvest, although better than the previous year, was only moderate. The yield from the harvest was expected to last until around March to May 1974. The reasons for the reduced yield were lack of oxen for ploughing, lack of seed, and poor rainfall.

Results of a survey in Tigray showed that of the rural population the very poor—those without land or livestock—were receiving only about half their required dietary intake, less than 1,000 kilocalories per adult per day and 50 kilocalories per kg per day for children; only one meal a day was usually being eaten. The major part of the population, the farming community, was faring a little better, relatively few of them being of less than 70% weight-for-height, and their dietary intake was marginally adequate, of about 1,500 kilocalories per adult per day, 75 kilocalories per kg per day for children; two meals a day were generally being eaten. The salaried (5–10%) workers were receiving up to 3,000 kilocalories per adult per day.

There was a proliferation of idiosyncratic methods of assessment of nutritional status and much nonquantitative subjective data. Standardisation of survey technique is important and perfectly feasible; nutritional survey methodology is an established aspect of nutritional science⁴.

Principles of nutritional surveys. In a situation of developing food shortage time-consuming detailed investigation before action may be unacceptable and crude rapid estimates may be required. Once action is established, aid and more thorough survey work can be concurrent, and indeed survey data is of vital importance in monitoring the effectiveness of the aid.

The primary environmental influences on nutritional status are dietary intake and the occurrence of disease; other

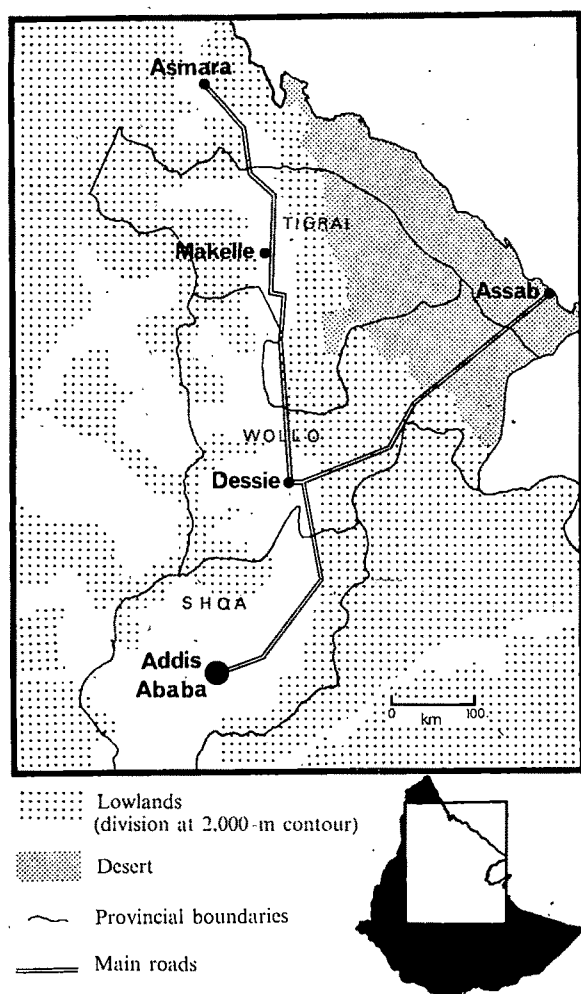


Fig. 1 Northern Ethiopia, showing area known to be affected by the drought in 1973. Highlands are unshaded.

factors—economic, agricultural, social and so on—necessarily exert their effect through either the dietary intake or the disease pattern (see Fig. 2). The nutritional status of the community defines the situation at a point in time but gives no idea as to trends. Current dietary intakes, food stocks and potential supplies provide the primary information as to the likely immediate trend of the nutritional status. The factors affecting nutritional status, shown in Fig. 2, also indicate the points at which action can be taken. Short term efforts, as in relief, are intended to influence dietary intake and disease patterns directly, by feeding programmes and public health measures. Longer term development work attempts to tackle the basic causes of malnutrition.

Nutritional status. The two characteristics of protein-energy malnutrition (PEM), wasting and oedema (associated with hypoalbuminaemia) require separate assessment. The wasting component of PEM is readily determined by anthropometric methods. Weight-for-height and height-for-age measurements ideally define both wasting and stunting⁵ but for a rapid assessment in an acute situation weight-for-height is the most useful measurement. The level of 70% weight-for-height is frequently taken to be approximately equivalent to the conventional 60% weight-for-age level used to define marasmus⁶. The risk to development of oedema (preclinical kwashiorkor) cannot be assessed by anthropometry; blood samples and determination of serum albumin values are required⁷.

Examination for clinical signs of PEM, together with anthropometry, defines the incidence of severe PEM in the community. The most relevant clinical signs are pitting oedema, generally appearing first on the dorsum of the foot,

and severe wasting, detected on the buttocks as 'baggy pants', when the skin hangs in loose folds; the appearance of this sign occurs at around 70% weight-for-height. Vitamin and mineral deficiencies are assessed by clinical examination.

Dietary intakes. Quantitative estimates of dietary intakes can only be obtained by home visits, in which the quantities of food eaten are weighed: nutrient intakes are then calculated from food tables^{8,9}. The two important parameters to consider are the total energy intake and the protein adequacy of the diet, estimated as effective protein concentration (Net dietary protein calories % or NDpCal%).

Community data. Questionnaires for home visits and for interviews with local officials and at health centres give data regarding current food stocks, livestock losses, expected harvest yields, population movements, patterns of disease and malnutrition and mortality rates.

Sampling. The validity and applicability of survey results depends on the sampling methods. Even in preliminary surveys, where the application of strict sampling techniques may be limited by the urgency of the situation, some attempt at randomisation is essential. Stratification by area and within the community can be based on existing demographic, geographical, agricultural and other data, and survey design can then concentrate the effort to manageable proportions. It is inadequate to try to gain an impression without selected home visits, since the most visible members of the community will tend to represent the extremes of nutritional status.

Conclusions from surveys. Such survey data define the current nutritional status of the community, the current dietary intakes and hence the trend in nutritional status of the current food stocks (livestock and crops), and the projected food supply in the immediate future.

The usefulness of survey data as a basis for planning depends on its quality. Rapid initial assessments not only become dated but are unlikely to be authoritative enough to support detailed planning.

Whether a situation requires special action (relief) depends on a consideration of the 'usual' situation in such areas. An assessment of a 'famine' situation perhaps depends on the extent to which the normal equilibrium has been disturbed, and in particular on the ability of the farming community to survive and to recover. The levels of hardship which are unacceptable and which should lead to relief being given require urgent consideration by aid-giving organisations.

Planning

Effective planning of relief is clearly essential but in an urgent situation it may involve particular problems of donor-recipient relationships. The decision to request aid may itself be difficult to take, and the recipient will rightly wish to direct the aid in accordance with its own priorities. These priorities may not agree with those of the donor countries, particularly if the donors are insensitive to local requirements. This can lead to poor coordination and a reduction in immediate and longer term gains. Responsibility for internal administration and coordination lies with the recipient. To ensure a maximum effectiveness, adequate planning and consultation are vital and prior consultation concerning Ethiopia's real needs has often been notably absent.

Successful planning requires that the problem be defined and priorities for action established; where possible these should also advance the longer term development aims. Only then can the appropriate responses be decided.

Nutritional responses

Food aid requested in 1973 was for both grain and 'high protein foods'. Quantities of grain were based on estimated requirements of 0.4 to 0.8 kg per head per day, supplying about 1,500 to 3,000 kilocalories per head per day. At 0.5

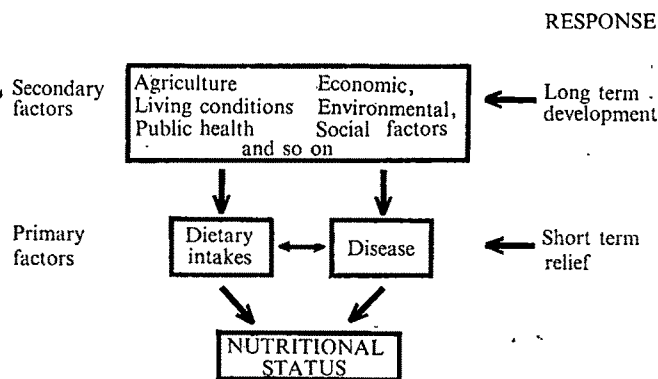


FIG. 2 Exogenous influences on nutritional status and levels at which efforts are directed to improve it.

kg per head per day 1,000 tons would supply 2 million man-days; thus a population of 1 million would require 15,000 tons a month, or 100 5-ton truck-loads a day. The main outside supplies were from international agencies, particularly World Food Programme, which consigned 20,000 tons of grain, of which 10,000 arrived in 1973 (enough to supply the estimated 2 million affected for 10 days, even if it could be distributed.) Further supplies of several thousand tons were redistributed in Ethiopia, and some hundreds of tons of specifically high protein food, designed originally as protein supplements, were also distributed.

It is estimated that about a quarter of the population of the affected areas will require food aid in 1974, and 150,000 tons of grain has been requested accordingly, on the basis of 0.4 kg per head per day; further supplies of protein foods have also been requested from international agencies.

The food supplied to the camps by the government provided 2,000 to 3,000 kilocalories per adult per day, as grain (mainly wheat and maize). At first it was difficult to prepare the food in a form regarded as suitable for main meals but the familiar 'enjera' (made from fermented grain) and 'wot' (a sauce containing legumes, spices and sometimes oil) were later introduced by the Ethiopian Nutrition Institute and were greeted with great enthusiasm.

The choice of foods imported and distributed by most external agencies showed an overemphasis on providing protein, regardless of energy intakes, particularly since the local protein supplement 'Faffa' was available to meet any extra protein requirement. Examples of foods supplied by foreign agencies are sweetened milk, protein biscuits, baby foods packed in individual portions, protein 'tonic' (containing 90% protein of unspecified origin) and, of course, dried skimmed milk. These were neither locally acceptable, nor suitable for treatment or prevention of malnutrition, nor indeed had most of them been requested. Further anomalies, such as the attempted further fortification of the local protein supplement with protein 'tonic', and the provision of exclusively protein supplements (in one case giving twice the protein and half the energy requirements) resulted from the misconception that the malnourished require only protein (and perhaps vitamin pills, which were also used incorrectly).

Principles of emergency food aid. In an acute food shortage, food aid is required

- to support the nonmalnourished population—'maintenance feeding', and
- to improve the nutritional status of the malnourished in the population, 'rehabilitation feeding'.

Both quantity and quality of dietary intake need consideration.

Distribution of supplies as widely as possible is clearly preferable to central cooking and feeding, in order to prevent migration and to enable the population to re-establish its normal food supply. Indeed the effective distribution of

supplies can be of critical importance in stimulating recovery from an emergency. The nutritional requirements for both maintenance and supplementary feeding are similar whether the supplies are distributed or prepared centrally, as in camps.

Maintenance. Maintenance feeding involves supplying either the total dietary intake for those completely dependent on relief supplies, such as nutritional refugees in camps, or in supplementing an inadequate diet when there are some resources locally available. Nutritional requirements for maintenance are derived from recommended intakes published by FAO/WHO¹⁰; these are for 3,000 kilocalories per adult per day, and 70–100 kilocalories per kg per day for children, depending on age. As an approximation 2,000 kilocalories per head per day (for example, from 0.6 kg of grain) irrespective of age would be a minimal allowance on a population basis. An effective protein concentration (NDpCal%) of 5% is recommended for all except children of up to 1 year and pregnant and lactating women, who require an NDpCal% of about 6–8. Cereals alone provide an NDpCal% of about 5 and are thus nutritionally adequate to support most of the population. Inclusion of legumes in the diet would increase the protein concentration (see ref. 11) and conform to normal dietary patterns: if 25% of the grain were replaced by legumes the NDpCal% would be at least 8 and thus the protein requirements of vulnerable groups would be met.

Thus the choice of foods for maintenance is based on supplying an energy intake of at least 2,000 kilocalories per head, with an effective protein intake that need be no greater than 8%. (The typical NDpCal% of high protein foods is at least 20%). The choice then depends on the availability, cost, and on the normal dietary patterns and thus acceptability of different foods. For Ethiopia there is no doubt that the main supplies that meet these criteria are cereals, such as wheat, and legumes (peas, beans and so on) with smaller quantities of vegetable oil and spices.

Rehabilitation. The nutritional requirements for improvement of nutritional status are higher than for maintenance, particularly to allow malnourished children to catch up lost growth. Increased dietary intakes can be achieved by 'supplementary' feeding of extra food for mild cases of malnutrition or by 'therapeutic' feeding for the most severely malnourished.

Energy intakes in excess of 100 kilocalories per kg per day for children, with a net NDpCal% of 8–10%, are required; in fact, rates of catch-up growth are approximately proportional to the energy intake above about 100 kilocalories per kg per day. When, as in Ethiopia, wasting is the main feature of the malnutrition, the overriding requirement is for increased energy intakes. If the total food intake is reduced extra protein is anyway wasted since it is used for energy. The key to supplying extra energy is to provide vegetable oil.

For supplementary feeding extra rations or meals should be provided for selected groups or individuals. For malnourished children it should be possible to increase energy intakes to 120–150 kilocalories per kg per day, with an NDpCal% of about 8. The choice of foods is similar to that for maintenance feeding, with greater emphasis of acceptability since the malnourished are frequently also sick and may have poor appetites, and the energy concentration of the diet should be increased with oil. In Ethiopia a sauce of high oil content, and thus high energy content, was readily constructed by a modification of the usual recipe. There is no advantage in using premixed foods for supplementary feeding; they have generally low acceptability and are expensive.

Therapeutic feeding requires organisation at an individual level and can only be carried out successfully within some centralised system. It is intended only for treatment of the most severely malnourished, who would normally be hospitalised if possible and who require intensive feeding in order to recover at all. Therapeutic diets are made up from fortified milk mixtures, having an NDpCal% of about 8 and providing

at least 150 kilocalories per kg per day for children. These diets are only applicable when a treatment system can be established, as was done in several camps in Ethiopia (see, for example, ref. 12); in this case use of a premixed diet is justified since the administration of correct and known quantities of nutrients is essential. If a system cannot be established for correct administration there is little value in using therapeutic diets.

Proper response

The nutritional consequences of the drought in Ethiopia have again (compare ref. 13) demonstrated the need for nutritional surveillance in assessing relief situations, for adequate planning and for appropriate responses in terms of food aid, based on an assessment of the situation, on a consideration of the normal dietary patterns and on sound nutritional principles. A rational and measured approach to relief is possible based on established principles and greater consideration of these could ensure that relief is more effective.

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Plate movement and continental magmatism

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Extensive magmatic and metamorphic events have occurred within the Earth's continental crust far from plate margins. During the last 800 million years Africa has only been host to such events during the breaks in the systematic motion of the African plate. This suggests that sublithospheric heat sources have little effect on a moving plate and can only produce recognisable effects in the upper crust if focused for long periods on the same part of the lithosphere.

SEISMICITY, tectonism and magmatism are now concentrated near plate margins. This may have been so throughout much of geological time. Within-plate continental magmatism and metamorphism are commonly believed to be associated with continental rifting¹⁻⁶ but only when rifting proceeds to the stage of transcontinental fracture is the association with plate margins clear.

This paper has three purposes. First, we emphasise that magmatic, tectonic and metamorphic processes have been and are active within continental plates and are by no means exclusively concentrated near plate margins, whether successful or abortive. Second, we note the circumstances in which this activity has occurred, using as an example the magmatic, metamorphic and palaeomagnetic history of a single continental mass, Africa, during the past 800 million years (m.y.). Third, we suggest that this behaviour is to be expected on the basis of thermal convection models of plate motion.

Magmatic and metamorphic activity in Africa

A wide range of igneous and metamorphic rocks are suitable for radiometric age determination. A plot of frequency of radiometric dates against time (Fig. 1) can therefore be used to indicate fluctuations in the intensity of thermal activity in the upper crust, though this cannot be regarded as an accurate quantitative measure. Nevertheless, Fig. 1 shows clearly that within the past 800

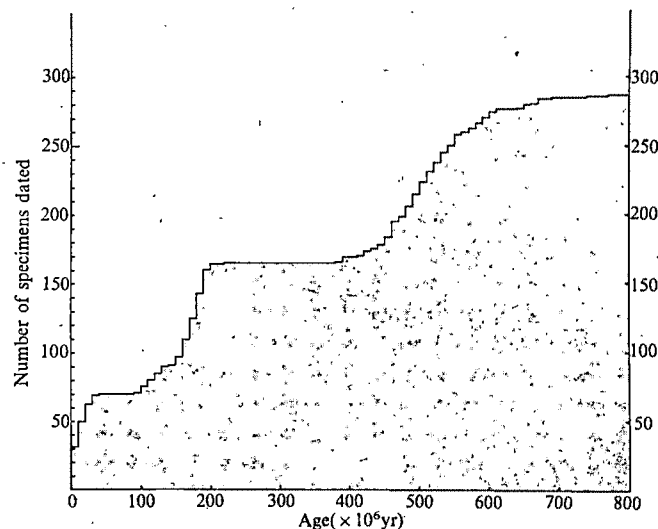


FIG. 1 Cumulative histogram of African radiometric age determinations for the period 0-800 m.y. BP.

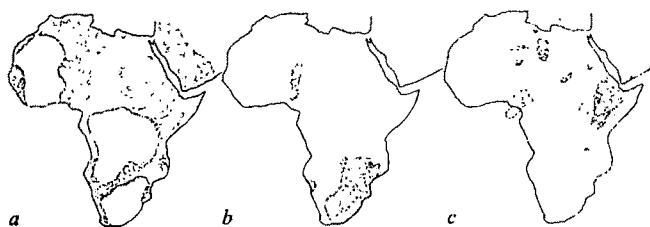


FIG. 2 a, Regions (stippled) of Africa which have been affected by the pan-African (650–400 m.y.) tectono-thermal event (after Clifford⁷). b, Areas (stippled) of Mesozoic igneous activity (200–100 m.y.) (after Cox⁸ and Black and Girod⁹). c, Areas of Tertiary to Recent volcanic activity 25 m.y.–present (after Le Bas¹⁰ and Gass¹¹).

m.y. Africa has been the site of three main thermal episodes in the approximate time ranges 650–400, 200–100 and 25–0 m.y. before present (BP). The areas affected by these events are shown in Fig. 2. Magmatic or metamorphic activity between 400 and 200 m.y. BP and between 100 and 25 m.y. BP is sparse^{7–11}.

Pan-African tectono-thermal event¹². Igneous and metamorphic rocks over most of Africa (Fig. 2a) yield K–Ar ages in the range 650–400 m.y. BP. Metamorphic rocks are the more abundant and their K–Ar ages record a thermal imprint on host rocks which Rb–Sr and U–Pb analyses indicate to be much older¹³. Kennedy¹² recognised that progressive regional metamorphism, ophiolite sequences and molasse-type sedimentation, which commonly characterise orogeny, occur only in restricted areas, such as the Lufilian¹⁴ and Pharusian^{15,16} chains in central and western Africa respectively. Elsewhere the event occurs as thermal reactivation of much older basement with extensive metamorphism and production of granitic magmas but little or no deformation. Even, however, if the Lufilian and Pharusian chains were the sites of pan-African subduction the amount of closure was small if palaeomagnetic evidence that Africa maintained the same approximate configuration throughout is accepted¹⁷.

Mesozoic magmatism. Between 200 and 100 m.y. BP magmatic activity was intense and widespread in southern^{18,19}, western and possible north-eastern Africa (Fig. 2b). For southern Africa, Cox¹⁰ concluded that igneous activity took place throughout the 200–100 m.y. time range and calculated that the erupted lava once covered 1.4×10^6 km². These rocks are mainly basaltic, but silicic varieties also occur and these may have been produced by anatexis of sialic crust¹⁰. Rocks of similar age in West Africa are predominantly silicic with only minor basic volcanic representatives. In both regions the activity was at its greatest between 200 and 180 m.y. BP and from then on generally waned, with occasional resurgences, until it ceased about 100 m.y. BP. Although this activity heralded the rupture of Gondwanaland, magmatism was not restricted to regions close to eventual plate margins. On the contrary, the bulk seems to have occurred within continental regions, which remained unified throughout.

Late Tertiary to Recent magmatism. Except in Libya where some volcanic rocks date at 40–50 m.y. BP, and Ethiopia where restricted volcanism might possibly have persisted throughout the Tertiary, rocks of magmatic origin in the age range 100–25 m.y. BP are extremely rare. Volcanic rocks with ages of less than 25 m.y. are, however, abundant (Fig. 2c). They are usually found where the underlying and commonly peneplain crystalline basement has been updomed, in places by as much as 3 km (ref. 11). Uplift usually preceded volcanism. Many of these areas of uplift and volcanism, such as Tibesti, the Hoggar and Air massifs, and the Bayuda and Jebel Marra, are completely within the African plate and any fracturing does not extend far beyond the limits of uplift. In eastern Africa the areas of

uplift around the Red Sea and in Ethiopia, Kenya and Tanzania overlap, and the resultant fracture zones join to form the East African Rift System. Beneath these rifts, sialic crust has been markedly attenuated although complete lithospheric rupture has only occurred under the Red Sea and Gulf of Aden. In every case the initiation of uplift and volcanism took place beneath what was then the unified Afro-Arabian lithospheric plate, and constructive plate margins only developed as events progressed.

Apparent polar wander

The apparent polar wander path for Africa since 750 m.y. BP^{17,20,21} is reproduced in Fig. 3. Many of the data were derived from the igneous rocks discussed in the preceding section and are thus well suited to the comparisons discussed here.

The palaeomagnetic poles derived from the Mbozi complex in Tanzania (743 ± 30 m.y.) and pre-Nama dykes in south-western Africa (653 ± 70 m.y.) both lie near the present pole. So also do well defined poles from several less well dated rock bodies in the Sudan, Tanzania and Zambia. It therefore seems that around 700 m.y. BP the pole (which is the South Pole) remained near the present North Pole for about 100 m.y., although age uncertainties prevent a positive conclusion.

Poles calculated from many African rocks ranging in age from 630 ± 24 m.y. to late Ordovician (approximately 450 m.y.) lie in or near western Africa. Although the precise form of this part of the polar wander path is not clear, the concentration of poles in this general area is established. It can therefore be inferred that the pole shifted from its position at 700 m.y. during the late Precambrian. The intervening polar path is, however, strewn with a number of pole positions derived from nominally Cambrian rocks from South America and the shift in question might therefore be as late as mid-Cambrian.

The polar path then moves to the vicinity of South Africa. The date of the shift is uncertain and has been variously estimated as late Ordovician²², Siluro-Devonian²³

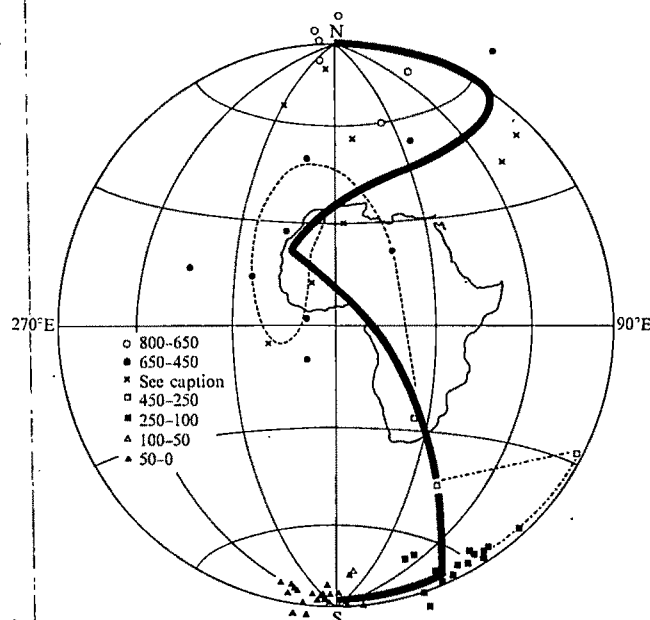


FIG. 3. Palaeomagnetic apparent polar wander path for Africa (thick solid line) from 750 m.y. BP to the present; data from compilations^{17,31,39,40} and recent original papers^{28,41,42}. Elaborations of parts of this path suggested by Piper *et al.*¹⁷ (---), and McElhinny *et al.*²¹ (·-·-) are not well substantiated. South American poles, supposedly of Cambrian age, rotated for closure of the South Atlantic, are denoted by x.

and Devonian^{24,25,26}. During the mid-Carboniferous²⁷, the pole is known to have moved away from South Africa, and therefore the estimates of the time for which it remained in that area range from only a few million years to rather more than 100 m.y.

Data from all parts of Africa except the north-east, confirm that the pole reached the vicinity of 60°S, 85°E at the beginning of the Mesozoic and remained there, essentially stationary, until about 110 m.y. BP (refs 21 and 24).

It essentially reached its present position by 40 m.y. BP, and it has stayed there ever since²⁸. This is reflected in the cluster of poles near the South Pole, shown in Fig. 3. The fact that their mean is some 5° from the geographic pole may be because of the inadequacy of data or persistent non-dipole elements in the palaeomagnetic field, rather than because of any true polar wander or plate motion²⁹⁻³¹.

There were thus at least three periods of time within the past 750 m.y. during which the palaeomagnetic pole did not move systematically relative to Africa, but instead either stopped or moved circuitously (Fig. 3). These periods occurred around 500 m.y. ago (at least 530-450 m.y. BP and perhaps as long as 630-400 m.y. BP), from 220 to 110 m.y. ago and during the past 40 m.y. Two other possible pauses in apparent polar wander, around 700 and 350 m.y. ago, have also been mentioned but the evidence for them is less secure. It is incorrect to translate apparent motion of the palaeomagnetic pole relative to a crustal plate, into direct estimates of the speed of the plate motion. To do so requires complete knowledge of the tectonic rotation poles, which describe the motion of the plate through time. Nevertheless, it seems realistic to equate episodes of rapid apparent polar wander with episodes of rapid plate motion. Similarly, it can be presumed that a plate has remained essentially stationary during an interval for which all palaeomagnetic poles are statistically indistinguishable.

Plate motion and thermal activity

The three major African igneous and metamorphic episodes coincide quite precisely with pauses in the systematic motion of the African plate (Fig. 4). In the case of the interval 600 to 400 m.y. the scatter of palaeomagnetic data is such that random or circuitous motion within about 2,000 km of the calculated mean position cannot be ruled out. But for the later two episodes the data suggest that motions of more than a few hundred kilometres are improbable. Each pause in systematic motion follows a period of substantial shift, and is succeeded by a new shift in a different direction. Despite the statistical uncertainties in radiometric dating and in palaeomagnetic estimation of latitude, and the impossibility of determining ancient longitude from palaeomagnetism, this remarkable time correlation is unlikely to have arisen by chance.

The evidence thus suggests that changes from one regime of plate motion to another were not accomplished rapidly. During the transitions, motions were either more complex or ceased altogether. This conclusion is intuitively sensible considering the enormous mass inertia of the system and has been advanced⁴³ to explain a correlation between bends in the Canadian polar wander path and orogenic events.

It also seems that extensive magmatism and metamorphism within the African plate only developed during these transitional periods. This might be anticipated when the enormous thermal capacity of the system is considered. From the general character of the global geological record it is clear that the lower crust and uppermost mantle are normally below their solidus temperatures. As the heat produced within the continental and oceanic lithosphere accounts for only about 65% and 3% respectively of the observed surface heat flow³², the fundamental requirement for magmatism

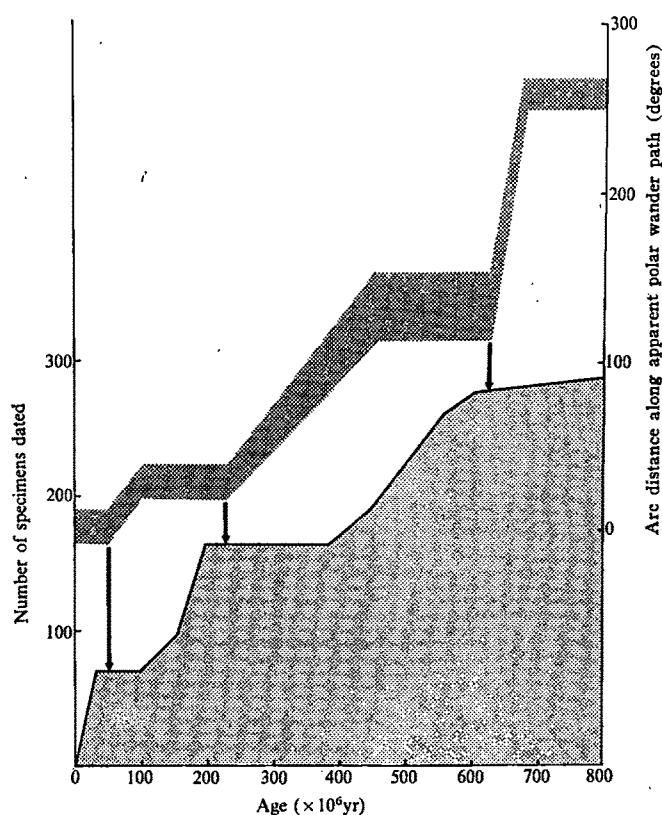


Fig. 4 Above, schematic representation of the erratic character of apparent polar wander (generalised from Fig. 3). Below, simplified cumulative frequency histogram of radiometric age determinations (based on Fig. 1). Vertical lines emphasise the correlation between the breaks in apparent polar wander and the start of thermal activity.

and metamorphism at high levels in the crust is a substantial heat source beneath the lithosphere. The fact that these effects are regionally restricted at particular times indicates that such sources are unevenly distributed. Our observations also imply that the heat sources have maximum effect when they are applied beneath the same region of lithosphere for many millions of years. Only then does large scale mantle or crustal anatexis occur. These in turn give rise to thermal metamorphism within a few kilometres of the surface. Whether the heat sources begin as localised hot spots of asthenospheric or deeper origin^{3,4,33-35}, or as more extensive asthenospheric upcurrents of a nascent plate-driving system, is not critical in this context. It is, however, pertinent to note that the ultimate source of the Mesozoic Karroo magmatism might have been as deep as 500 km (ref. 19).

For the thermal source and the target to be fixed relative to each other, either both must move together³⁶ or, as is contended here both must remain stationary with respect to the rotation axis of the Earth.

The identification of linear age trends along particular lines of igneous activity³⁷ does not invalidate our main conclusions. Such trends are rare, have been disputed^{36,38} and in any case are short when compared with the dimensions of the igneous provinces as a whole.

If thermal conduction were the only heat transfer mechanism, there would be a time lag of the order of 10^8 yr before sublithospheric thermal events permeated to the surface. The effects could be localised and accelerated by convective heat transfer in the asthenosphere and by upward magma migration. Although it is tempting to suggest that time lags are in fact observed between apparent cessation of African plate motion at 220 and 40 m.y. and the surges of subsequent igneous activity at 200 and 25 m.y. respectively (Fig. 4), this inference is probably unjustifiable on the

evidence available. Certainly no such effect is evident for the pan-African event.

Nevertheless, our analysis demonstrates that widespread continental igneous activity could be the result of stationing a plate over a heat source of a kind which could, potentially, cause continental breakup. It is suggested that thick continental lithosphere will not inevitably break in response to such heating. Instead it may become locally elevated, the thermal gradient may become enhanced and extensive anatexis may be produced as well as widespread thermal metamorphism of pre-existing rocks of the upper crust.

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Further evidence of Lower Pleistocene hominids from East Rudolf, North Kenya, 1973

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Twenty new hominid specimens were recovered from the East Rudolf area in 1973. New evidence suggests the presence of at least three hominid lineages in the Plio-Pleistocene of East Africa.

THIS is a report of the 1973 field season at East Rudolf, Kenya, where the East Rudolf Research Project (formerly Expedition) has now concluded its sixth year of operations. Eighty-seven specimens of fossil hominid were collected¹ from the area during 1968-72; a further twenty specimens were recovered between June and September 1973 from the Upper, Lower and Ileret Members of the Koobi Fora Formation². Exploration to the south of Koobi Fora was begun in 1972 and continued in 1973. No hominids have yet been found

in the limited exposures of the Kubi Algi Formation. A notice of two specimens—KNM-ER 1510 and 1590—that were previously¹ mentioned only by number, is included in this report. The 1973 hominids are not here attributed to genera as there are still no clear generic diagnoses available for fossil hominids. With a few exceptions, previous attributions for the East Rudolf hominid collection remain satisfactory.

Archaeological investigation during 1973 was extended under the direction of G. Ll. Isaac, with J. C. W. Harris who conducted major excavations at several sites in the Upper Member of areas 130 and 131. Limited excavation, but extensive prospecting, in the Lower Member produced sufficient results to support further searching for artefacts below the KBS Tuff.

TABLE 1 1973 hominid collection from East Rudolf

KNM-ER No.	Specimen	Area	Member
1800	Cranial fragments	130	Lower
1801	Left mandible, P ₁ , M ₁ , M ₂	131	Lower
1802	Left mandible, P ₁ -M ₂ and right P ₃ -M ₂	131	Lower
1803	Right mandible fragment	131	Lower
1804	Right maxilla, P ₃ -M ₂	104	Upper
1805	Cranium and mandible	130	Upper
1806	Mandible	130	Upper
1807	Right femur shaft	103	Upper
1808	Associated skeletal and cranial fragments	103	Upper
1809	Right femur shaft	127	Lower
1810	Proximal left tibia	123	?Lower
1811	Left mandible fragment	123	?Lower
1812	Right mandible fragment and left I ₁ and M ₁	123	Lower
1813	Cranium	123	?Lower
1814	Maxillary fragments	127	Upper
1815	Right talus	1	Upper
1816	Immature fragmented mandible	6A	Upper
1817	Right mandible	1	Upper
1818	I ¹	6A	Upper
1819	M ₃	3	Upper
1820	Left mandible with M ₁	103	Upper

During the palaeontological survey, which was supervised by J. M. Harris, all identifiable fragments from certain horizons were collected; new species were recorded and some primate remains were recovered during a limited survey of the Kubi Algi Formation. A detailed account of the East Rudolf fauna will be presented upon conclusion of current studies, but there are clear indications that at times the palaeoenvironment differed from that of the lower part of the Shungura Formation of the Omo Valley in Ethiopia.

In the geological studies, emphasis was placed on microstratigraphy and palaeoenvironmental reconstruction. B. Bowen supervised a study of the Lower and Upper Members of areas 130 and 131 which included confirming the stratigraphic relationships of the cranium KNM-ER 1470. The complete section of the Koobi Fora Formation exposed in area 102 was studied by a group from Dartmouth College, New Hampshire, under G. Johnson. A. K. Behrensmeyer completed a preliminary geological investigation of the hominid sites, noting depositional environments and possible association of fauna; further studies are planned.

I. Findlater extended mapping of tuffaceous horizons to the south of Koobi Fora and collected samples for isotope dating. A series of dates has been obtained from material collected during 1972 (unpublished work at Miller, Findlater, Fitch and Watkins). Palaeomagnetic studies complement those of 1972 and there are sufficient data for internal correlations to be made³.

Hominid collection

Specimen KNM-ER 1590, reported previously¹, consists of dental and cranial fragments which were collected from area 12, some metres below the KBS Tuff. Both parietals, fragments of frontal and other pieces of cranial vault, the left deciduous c and dm², the left and right unerupted C, P³ and P⁴, and the erupted left and right M¹ and left M² were recovered. Although the cranium is immature, it was large with a cranial capacity as great as that determined for KNM-ER 1470. The parietals may show some deformation but, in any event, they suggest that the cranium was wide with a sagittal keel.

KNM-ER 1510, also reported previously¹, includes cranial and mandibular fragments. The specimen is poorly mineralised and further geological investigation at the site indicates a Holocene rather than an early Pleistocene provenance as originally thought.

The 1973 hominids and their stratigraphical positions are listed in Table 1. Specimens from area 123 are rare, and their stratigraphical position relative to the Upper and Lower Members of the Koobi Fora Formation needs clarification.

A well preserved mandible (Fig. 1), KNM-ER 1802, was discovered by J. Harris *in situ* below the KBS Tuff in area 131. The dentition is only slightly worn, and fragments of both M₂ crowns suggest that death occurred before full eruption. The canines and incisors are represented by roots and by alveoli filled with matrix. The mandible shows some interesting features—moulding of the mandibular body, absence of a strong post-incisive planum, the development of a slight inferior mandibular torus and the distinct eversion of the mandibular body when viewed from below.

A weathered mandible, KNM-ER 1801, bears some resemblances to KNM-ER 1802, but its worn dentition and loss of surface bone prevent direct comparisons. The relative proportions of the molars and premolars may have been exaggerated by interstitial wear.

A crushed maxillary fragment, KNM-ER 1804, with P³-M² preserved was discovered by R. Holloway. The teeth are complete but worn.

A skull (cranium with associated mandible), KNM-ER 1805, was discovered by P. Abell *in situ* in the BBS Tuff complex in area 130. The specimen is heavily encrusted with a hard matrix and will require careful preparation before its morphology is revealed. Comments here are thus preliminary. The cranium is in pieces which fit together. After preparation, it should be possible to determine the endocranial capacity; at present, a volume of 600–700 cm³ is suggested. The supraorbital region, much of the face and the greater part of the basi-cranium have not been preserved. The postorbital region is preserved and the minimum breadth is approximately 90 mm. No distinct temporal lines cross the frontal area although they can be discerned and are still apart at the bregma. There are distinct parasagittal crests. The nuchal attachments are very distinctive and protrude to form a wide bony shelf. The palate is intact; all the teeth are preserved except for the right P⁴ and the left I¹. The mandible, small and distinctly robust, is represented by both sides of the body but, except for the right M₂ and M₃, the tooth crowns are missing. The ascending rami are not preserved. The right M₃ and M₂ are well worn but small. The upper dentition shows wear on all the teeth, including M³.

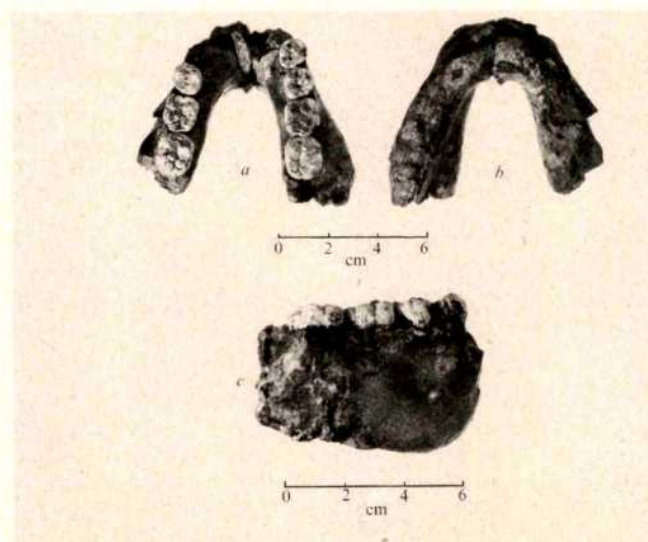


FIG. 1 Mandible, KNM-ER 1802. a, Superior view; b, inferior view; c, right lateral view.

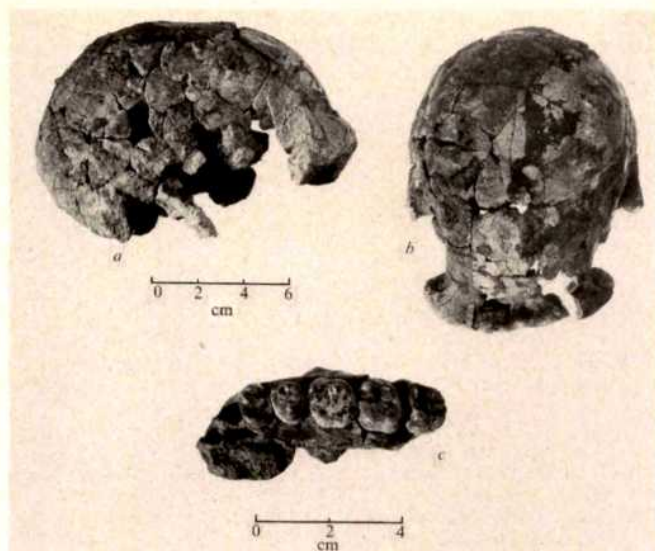


FIG. 2 Cranium, KNM-ER 1813. *a*, Right lateral view; *b*, superior view; *c*, occlusal view of left side of palate.

A large mandible, KNM-ER 1806, was discovered by Meave Leakey at the same site and horizon as was KNM-ER 1805. There are no tooth crowns preserved and the ascending rami are missing in this otherwise complete specimen. The mandible is typical of the large East Rudolf hominid that I have previously attributed to *Australopithecus*.

A fragmented specimen, KNM-ER 1808, was discovered in area 103 by Kamoya Kimeu. The specimen includes maxillary and mandibular teeth, cranial and mandibular fragments, a fragment of atlas vertebra, the distal half of a femur lacking the condyles, a large segment of humerus and other postcranial fragments. There is little doubt that the various pieces are from one individual and further sieving and excavation will be undertaken in the hope of recovering more material.

A cranium, KNM-ER 1813 (Fig. 2), was discovered *in situ* by Kamoya Kimeu in area 123. The specimen was fragmented but has been partially reconstructed. Plastic deformation is evident. The cranium is partly covered with a thin coat of matrix and considerable preparation is needed before a detailed description can be attempted. The endocranial volume is likely to be small; a figure of approximately 500 cm³ is suggested on the basis of comparative external measurements. Other interesting features include the curvature of the frontals, a postglabella sulcus and the small dentition. The maxilla has well preserved teeth, P³-M³, on the left side, but on the right side only the tooth roots and the complete crown of M³ remain. Both canines and lateral incisors are present but the central incisors seem to have been lost before fossilisation. Both sides of the maxilla fit together to give the form of the dental arcade. The right maxillary fragment includes the malar region and connects with the lateral margin of the right orbit.

Other specimens recovered during 1973 are listed in Table 1 and will be described in detail after studies are completed.

Significance of the 1973 collection

In previous reports^{1,4-7}, the East Rudolf hominids were assigned to *Australopithecus*, *Homo* or indeterminate (the last category included both very fragmentary specimens and those of uncertain taxonomic rank).

The East Rudolf specimens that have been attributed to *Australopithecus* span a period of time from 3 million years to just over 1 million years with apparently little morphological change. This form is likely to be the same species

as *A. boisei*⁸; it also shows similarities with *A. robustus* from southern Africa. A Pliocene origin is suggested for this specialised group.

Specimens attributed to *Homo* have been recovered from deposits covering a similar time span, but these show greater morphological variability. Those recovered from the Ileret Member seem to differ from those recovered from the Lower Member of the Koobi Fora Formation. The suggestion that a large brained, fully bipedal hominid was living at East Rudolf 3 million years ago was put forward after the 1972 discoveries⁷. This point of view is supported by the cranial fragments, KNM-ER 1590, also from below the KBS Tuff, and this specimen is provisionally attributed to *Homo*.

The 1973 collection from East Rudolf raises many questions. The new mandible, KNM-ER 1802, could be considered as belonging to the same genus and species as KNM-ER 1470 and 1590. There are striking similarities between the dental characters of KNM-ER 1802 and some specimens from Olduvai Gorge such as the type mandible of *Homo habilis*, OH 7. Although the suggested cranial capacity for *H. habilis* is appreciably smaller than that determined for KNM-ER 1470, the discrepancy may be due to the fragmentary material upon which the former estimates were made. I consider that the evidence for a 'small brained' form of *Homo* during the Lower Pleistocene is tenuous.

The cranium, KNM-ER 1813, may prove to be quite distinct from the robust australopithecines and from *Homo*, as represented by KNM-ER 1470. The dentition is 'hominine', yet the cranial capacity appears small. The cranium has some of the features seen in the gracile, small brained, hominid *Australopithecus africanus* Dart, from Sterkfontein.

I have previously questioned the validity of a distinct gracile species of *Australopithecus*⁶, but this new evidence reopens the possibility of its existence. Some authors^{9,10} have suggested that *Homo habilis*, particularly OH 24, shows features typical of *Australopithecus africanus*. My suggestion here, that *H. habilis* may have affinities with KNM-ER 1470 and 1590, refers only to OH 7 and OH 16. Features of the calvarium of OH 24 show similarities with KNM-ER 1813. The size and morphology of the teeth of the two specimens are alike and the cranial capacities may also be comparable^{11,12}.

The skull KNM-ER 1805 is undoubtedly important, but its interpretation is enigmatic. Its relatively large cranium bears sagittal and nuchal crests but has small teeth; this combination is in contrast to all the specimens previously recovered from East Rudolf.

In any consideration of the affinities of the East Rudolf hominids, the question of sexual dimorphism must not be overlooked. There does seem to be evidence for quite marked sexual dimorphism in one hominid group as demonstrated by the East Rudolf crania, KNM-ER 406 and 732⁵. Unfortunately both crania lack teeth so that the dental characteristics of the alleged female are far from clear.

The possibility of more than two contemporary hominid lineages in the Plio-Pleistocene of East Africa may now have to be recognised, whereas previously one, or at most, two forms were assumed. The attribution of isolated teeth may thus become even more difficult than it is now. Postcranial identifications likewise may be difficult, although the proximal femoral material continues to suggest a morphological dichotomy.

I suggest the following as a basis of nomenclature for Plio-Pleistocene hominids. One genus would include much of the material currently referred to *Australopithecus robustus* and *A. boisei*. A second genus would incorporate many of the gracile specimens from Sterkfontein presently referred to *A. africanus*, perhaps certain specimens from East Rudolf including KNM-ER 1813, and possibly some from Olduvai such as OH 24. A third genus, *Homo*, would incorporate specimens such as KNM-ER 1470 and 1590 from East Rudolf

and possibly OH 7 and OH 16 from Olduvai. Some material from South Africa might also be considered within this last category together with later specimens from Olduvai and East Rudolf. The unusual mandible KNM-ER 1482¹, together with the specimen from the Omo area referred to *Paraustralopithecus*¹³ and certain other specimens from Omo which are contemporary with the three groups just mentioned, could be considered a fourth form—a remnant of an earlier population that disappeared during the early Pleistocene. All these forms may be traced back well beyond the Plio-Pleistocene boundary.

These remarks are necessarily speculative. A more detailed review of hominid systematics is being prepared in collaboration with B. A. Wood. The wealth of data now available presents a new era in the study of early man. The complexities of dealing with the enlarged sample are challenging, and isolated studies on specific specimens must be replaced by exhaustive studies on all the fossil hominid evidence.

I should like to express appreciation for the financial backing provided by the National Geographic Society, the National Science Foundation, the W. H. Donner Foundation and others. The support and encouragement of the National Museums of Kenya and the Kenya Government

made the research possible. Members of the East Rudolf Research Project are too numerous to thank individually but all play a part in a successful field season and are thanked along with those who made important discoveries. I would also express thanks to my wife Meave who, as always, provided invaluable assistance both at the museum and in the field.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Years of peak astronomical tides

THIS investigation stems from an obscure statement by the late D. H. Macmillan in a semi-popular book on tides¹. He stated (page 48) that the years when the tide-raising forces reach their greatest peak values occur at intervals of very roughly 16 centuries, and gave a sequence of dates starting with 3500 BC and ending with 1433 and 3300 AD. He did not describe the exact terms of the calculation but merely suggested that they involved coincidence of perihelion (closest approach of the Earth to the Sun) and zero solar declination (Sun crossing equator), which is in fact impossible within the given range of dates. (The longitude of perihelion crosses the equator at intervals of about 105 centuries and will next do so in 6581 AD). Further, if one computes the lunar and solar elements for 3300 AD, for example, one finds that they are not particularly favorable for large tides.

To clarify the situation I must first resolve a paradox. By simple consideration, the greatest tide-raising forces must occur when the Moon and the Sun are in conjunction with the Earth and are at their closest respective distances. But it is also common knowledge that the greatest 'perigee-spring tides' (spring tides coinciding with a close approach of the Moon) occur at the equinoxes when the Sun crosses the equator, which as mentioned above cannot coincide with the closest approach to the Sun around the present millennium. The solution lies in the fact that the equinoctial condition maximises only the semi-diurnal components of the tide at the expense of the diurnal components. Since most parts of the ocean have a magnified response to the semi-diurnal frequencies, one must therefore look to the equinoxes

rather than perihelion for the greatest ocean tides. Then, all the major 'harmonic constituents' μ_2 , $2N_2$, N_2 , M_2 , L_2 , S_2 , K_2 are nearly in phase, and the only loss due to the slightly more than minimum distance of the Sun would be an unfavourable phase for the constituent T_2 , which is practically negligible because it has a typical amplitude of 2 or 3 cm of water (about 6% of the mean solar tide). On the other hand, if we are more interested in the maxima of the tide-raising forces themselves, or in movements of the Earth's crust which respond to them more directly than the ocean, then position relative to the equator becomes irrelevant and we should confine attention to the times of perihelion. The dates when both types of maxima occurred are considered separately below.

I restricted the computations to the years 1 to 4000 AD. Outside this range, the accepted formulae become unreliable and interest in the problem diminishes. For the times of peak tide-raising forces, the computer was asked to select the years for which the following conditions all occurred simultaneously: (a) Earth at perihelion, (closest to Sun); (b) longitude of Moon's perigee within a small angle ϵ of perihelion or of its converse (aphelion); and (c) longitude of Moon's ascending node within ϵ of perihelion or of its converse. The Moon's actual (mean) longitude was also computed at the given times, to check whether the angular limits were not violated by the time needed to adjust to the nearest appropriate conjunction with the Sun (the conjunction very near to perigee). Standard formulae, as found in the *Astronomical Ephemeris*, were used for the four relevant mean longitudes. With $\epsilon = 5^\circ$, perihelion satisfied the above conditions in only the following years: 1340, 1433, 1526, 1619, 3182, 3275, 3368 and 3461.

The appearance of the year 1433 shows that this was the type of calculation behind Macmillan's dates, but 3300 does not appear and there is no suggestion of a 1,600 yr cycle. On

the contrary, the salient features are sequences of exactly 93 yr occurring in groups of four in alternate millennia (also confirmed by tentative calculations for the first millennium bc). The 93 yr period arises because it is close to 5 times the 18.61 yr period of revolution of the Moon's nodes and 10.5 times the 8.85 yr period of perigee—condition (b) permits half periods. But, as the basic periods are strictly incommensurate, one cannot expect any exact cycle of repetition.

The configurations on most of the eight occasions above are, however, somewhat spoilt by the longitude of the Moon itself. If the Moon's 'anomaly' (its angular distance from perigee) is more than about 60° at the given instant, an adjustment of 5 to 15 d is required to define the optimum position, during which time the Sun moves by as many degrees as days. The only cases for which the Moon's anomaly and its angular distance from the Sun are less than 60° are in the years 1340 and 3182. Only in these two years does the peak tide-raising force truly occur within 5° of perihelion, perigee and a node.

Turning now to the case of peak semi-diurnal tides, the relevant computations follow a similar pattern, except that in the descriptions of conditions (a), (b) and (c), 'perihelion' is replaced by 'equinox'. (That is, I take the Sun over the equator instead of at its least distance from Earth.) Since equinoxes occur twice as often as perihelion, the conditions tend to be satisfied more frequently. The interval of 4.4 yr between passages of the longitude of perigee past the equinoxes, (condition (b) has been observed in the study of extreme low (or high) predicted sea levels². It might be thought that condition (c) should be confined to the autumnal equinox, so that the 18.61 yr 'nodal modulation' gives the constituent M_2 its greatest amplitude, but in fact when the Moon passes the equator, M_2 is in phase with K_2 , which has a correspondingly reduced amplitude, and other 'nodal modulations' are similarly self-cancelling. So both equinoxes are equally valid in condition c.

With $\epsilon = 5^\circ$, the three conditions are satisfied on 27 occasions in 1 to 4000 AD. Only those for which the Moon is within 60° of the appropriate conjunction are shown in Table 1; for reasons stated earlier. V represents the vernal and A the autumnal equinox.

TABLE 1 Most likely dates of peak tides

135A	1020V 1113V 1745A 1922A	2192A 2732V 2825V	3002V
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This time no cyclic pattern emerges, either in Table 1 or in the complete list of 27 dates. Intervals of 93 yr occasionally appear (for example, 1020–1113 and 2732–2825) for the same reason as before, but the use of the fixed reference of the equinox instead of the slowly moving perihelion renders the 93 yr cycle less persistent.

The conditions for 1922 were particularly favourable, since the Moon was less than 1 d off 'change' (the instant when 'New Moon' commences) at the time of the equinox and the other relevant angles were also very small. Accurate computations of the lunar and solar ephemerides³ show that the peak semi-diurnal tide-raising force occurred 1922 September 21 0539 h GMT, with amplitude 1.8728 times the mean lunar amplitude (M_2) or 1.2782 times the mean 'spring' amplitude ($M_2 + S_2$). Actual ocean tides usually reach their peak a little later than the peak tidal forces, because of the peculiarities of their dynamic response. Among tide-gauge records from 1922 which are ready to hand, Brest reached a peak of $1.72 \times M_2$ range, ($1.28 \times$ mean 'spring' range) on the tide of September 22 p.m. to

September 23 a.m., while Newlyn reached $1.64 \times M_2$ range ($1.22 \times$ mean 'spring' range) on the same tide.

Dynamic response may well, of course, cause the tide at a given place to reach its ultimate peak in other years. Places with large diurnal components would again require a quite different set of conditions.

This subject has recently taken on a surprising topicality because of widespread press reports (some grossly exaggerated) of forecasts of unusually large tides for certain days in 1974. The situation is that on January 8 the Moon's perigee occurred within less than 2 h of Full Moon, while the Earth was within 5 d of perihelion. This is close to the conditions for peak tide-raising forces but the fact that the Moon's line of nodes was some 16° away from the Sun disqualifies it from my more rigorous conditions. Being some 72 d off the spring equinox, the (semi-diurnal) oceanic tides are not extraordinarily large and a glance through the tide tables of recent years confirms that equally high and low waters were forecast, for example, for 1966 and 1972. The Full Moons of February and March 1974 occur successively later than perigee, but the approach of the equinox of March 21 compensates to make the spring tides about equally high in all three months. A similar sequence occurs in July, August and September when perigee occurs near New Moon but this time the Sun is not unusually close. The year 1974 is perhaps remarkable for its number of fairly large tides but if one were to compute the occurrences of equal or greater tides in 4,000 yr, one would obtain a very long list.

I thank Mr C. R. J. Davey for calling my attention to the inconsistencies in Macmillan's account of the subject¹.

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Creation of an artificial lunar atmosphere

THE tenuous nature of the lunar atmosphere is maintained by rapid loss of gases released at the lunar surface. But would such rapid loss still occur if the density of the lunar atmosphere were greatly increased from its present value? Here I seek to answer this question by evaluating quantitatively the atmospheric loss mechanisms operating in the lunar environment. One conclusion is that if the density of the lunar atmosphere is increased, a point can be reached where loss occurs so slowly that it is negligible over human time scales (that is, exponential decay lifetimes are greater than hundreds of years). So the present lunar 'vacuum' is a fragile state that should be treated carefully if it is to be preserved or could be modified if so desired.

The present lunar atmosphere has measured surface number densities of less than 10^7 cm^{-3} (refs 1–3) and a total mass of approximately 10^4 kg . As a result the entire lunar atmosphere is a collisionless exosphere in which the constituents travel along ballistic trajectories between collisions with the surface. When they are re-emitted from the surface, a fraction have sufficient thermal energy to escape from lunar gravity. The exponential lifetime against such thermal escape is about 3,000 yr for atoms of mass 20 (ref. 4). A more efficient loss mechanism, however, is operating in the lunar environment^{2,4,5}. This involves the interplanetary electric

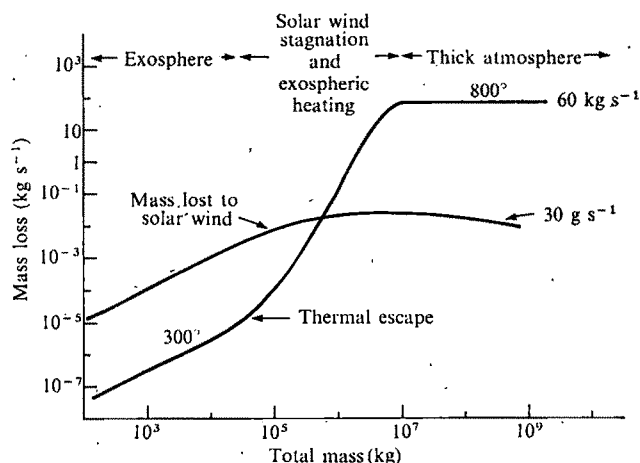


Fig. 1 Loss rates for an oxygen (mass 16 a.m.u.) atmosphere. Molecular oxygen has a smaller thermal escape rate but dissociation would occur in the upper atmosphere. The thick atmosphere thermal loss has probably been overestimated since I have assumed the entire planetary exosphere to be isothermal. Also, exospheric heating may not occur as rapidly as indicated. At a total mass of 10^8 kg the lunar atmosphere approaches a constant rate of mass loss. Temperatures are in K.

field resulting from the motion of the solar wind. An atmospheric ion formed through photoionisation by the solar ultraviolet or by collisional ionisation by the solar wind is accelerated initially in the direction of the interplanetary electric field. Half of the atmospheric ions are thus lost into space and half are driven into the surface. The time constant for exponential decay of the atmosphere by this process is determined by the ionisation lifetime (typically 10^6 to 10^7 s). Thus, gases added to the lunar exosphere are lost within weeks to months. This rapid loss has been confirmed by observations of Lunar Module exhaust gases by the Apollo Suprathermal Ion Detector Experiment^{6,7}. There is, however, a limit to the amount of atmosphere that can be swept away by the solar wind. If the atmosphere is dense, newly formed ions of atmospheric origin load down the solar wind flow and divert it around the planet. Thermal escape may then become the dominant loss mechanism.

A quantitative evaluation of these atmospheric loss mechanisms is shown in Fig. 1 for an oxygen atmosphere. The exospheric loss rate to the solar wind was calculated assuming that the total ionisation rate was 5×10^{-6} ion per atom s^{-1} and that half of the exospheric mass was on the dayside. The limit to mass lost to the solar wind for a thick atmosphere is taken as equal to the solar wind mass flux through the lunar cross section ($\sim 30 \text{ g s}^{-1}$), since critical mass loading of the solar wind occurs if ions are added at a rate comparable to the solar wind flow⁸. Venus and Mars each lose about 10 g s^{-1} to the solar wind^{8,9}, which is 1% (Venus) and 20% (Mars) of the mass flux of the solar wind through their cross-sectional area. The thermal escape rate⁴ was calculated with 300 K as a weighted average of the lunar surface temperature. Absorption of the solar wind and ultraviolet by a thick atmosphere results in exospheric heating and more effective thermal evaporation. As the atmospheric density is increased, however, the exospheric base rises above the surface and the mass lost to thermal evaporation becomes constant. Consequently, the lifetime increases linearly as the atmospheric mass increases. It is not yet certain to what density the lunar atmosphere can be increased; this problem is being investigated.

Figure 2 shows the ultimate atmospheric masses which result from various constant gas addition rates, Q . The effect of inducing a transition from an exosphere with rapid loss to a thick atmosphere with slow loss is illustrated by considering

the result when the gas source is shut off ($Q = 0$). The thick atmosphere decays with an exponential lifetime of several hundred years whereas the thin exosphere decays in a few weeks. It is estimated that a constant addition rate of approximately 100 kg s^{-1} is required to transform the lunar exosphere into the long lived atmosphere state. Although the total atmospheric mass would increase to 10^8 kg , the surface density would still be low compared to densities in the terrestrial atmosphere.

These considerations do not include possible loss of gases to the lunar surface. Adsorption of gases such as nitrogen oxygen and carbon monoxide by the lunar soil is reversible and permanent retention (chemisorption) generally does not occur¹⁰⁻¹³. The adsorptive capacity of lunar soils is typically only 10^{-4} to 10^{-5} g of adsorbate per g of soil, and the crystalline rock is probably a factor of 10 to 100 less adsorptive¹¹. Assuming that the upper 1 mm of the entire lunar surface fully adsorbs gases with a capacity of 10^{-5} , then the total capacity is $5 \times 10^8 \text{ kg}$. This is comparable to the estimated minimum mass needed to produce a long-lived atmosphere. Consequently, surface adsorption of gases would at worst result in a slight delay in atmospheric growth but would not act as a permanent barrier.

The significance of these quantities can be evaluated by examining ways of artificially adding gases to the lunar atmosphere. Each Apollo mission deposits nearly 10^4 kg of rocket exhaust into the lunar environment⁴. Since this is much less than the 10^8 kg needed to create a long lived atmosphere, exploration at the rate of several Apollo missions per year would seem to present no lasting hazard to the lunar environment. A permanent lunar base would possibly release gas at a rate equivalent to $10^{-2} \text{ kg s}^{-1}$ per man, assuming supply traffic equal to one Apollo mission month⁻¹ per man. Therefore small lunar colonies would probably not produce a long lived atmosphere. But even modest lunar exploration is likely to release gas faster than the present natural source rate of $\sim 20 \text{ g s}^{-1}$, resulting in a lunar atmosphere in which the gases of natural origin would be only trace components. Vigorous lunar exploration and colonisation could easily result in gas release rates greater than $10^{-2} \text{ kg s}^{-1}$ per man. Proposed activities such as placing factories on the Moon to avoid pollution of the Earth's atmosphere¹⁴ need careful evaluation to avoid lasting contamination of the lunar environment.

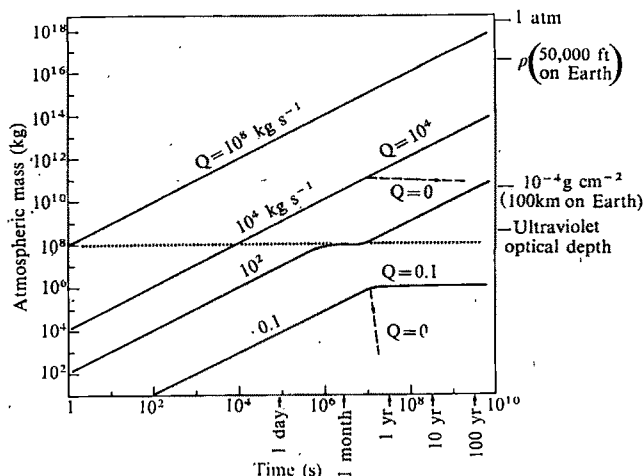


Fig. 2 Growth curves of the lunar atmosphere for various constant gas addition rates, Q . The transition to a long lived atmosphere occurs at a total mass of 10^8 kg which requires a rate of release of gas of about 10^2 kg s^{-1} . The present lunar atmospheric mass is approximately 10^4 kg and is maintained by a natural source of $\sim 20 \text{ g s}^{-1}$. Comparable densities in the terrestrial atmosphere are indicated. ---, Decay in total mass if the gas source is shut off

If one wanted intentionally to create an artificial lunar atmosphere, gases can be obtained by heating or vaporisation of the lunar soil. Approximately 25 MW is needed to produce 1 kg s^{-1} of oxygen by soil vaporisation. An efficient mechanism for gas generation is subsurface mining with nuclear explosives. K. Ehricke (in a North American Rockwell report) estimates that a 1 kton nuclear device will form a cavern approximately 40 m in diameter from which 10^7 kg of oxygen can be recovered. Application of this technique can easily generate enough gas (10^8 kg) to drive the Moon into the long lived atmosphere state.

The desirability of intentionally increasing the density of the lunar atmosphere is highly questionable, since the primary applications of a lunar laboratory involve utilisation of the present lunar 'vacuum'¹⁵⁻¹⁷. But the artificial generation of an atmosphere can be considered as another potential method for human modification of planetary environments¹⁸⁻²¹.

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Thermal emission of Saturn's rings and disk at $34 \mu\text{m}$

OBSERVATIONS from aircraft of Saturn's thermal emission do not resolve the flux component of the rings from that of the planet^{1,2}. Ground-based observations in the windows at 11 and $20 \mu\text{m}$, which resolve the rings and disk contributions, include only a small fraction of the thermal flux and are sensitive to greenhouse effects³⁻⁶. Using new filter techniques

for ground-based observations⁷, we have measured the relative thermal emission from the rings and disk of Saturn in the band from 29 to $43 \mu\text{m}$. This measurement provides an important constraint for separating the thermal properties of Saturn from those of its rings.

Ground-based photometry extending to $\sim 40 \mu\text{m}$ is possible from high altitude sites under dry weather conditions^{7,8}. The measurements between 29 and $43 \mu\text{m}$ reported here were made with the 224-cm telescope at Mauna Kea Observatory in March 1973. Additional measurements at 17 to $28 \mu\text{m}$ for normalisation purposes were recorded in September 1973. For convenience we refer to the band 29 to $43 \mu\text{m}$ (FWHM) as the $34\text{-}\mu\text{m}$ band, and the band 17 to $28 \mu\text{m}$ as the $20\text{-}\mu\text{m}$ band, although the effective wavelengths may depart significantly from these nominal values depending upon the source spectrum. In both spectral bands the ring and disk components were measured separately with a 9 arc s aperture centred on the disk and on the ansae at Cassini's division (the latter position subtended about equal areas of the A and B rings). The beam was sinusoidally chopped over a distance of 27 arc s along a direction aligned within a few degrees of Saturn's polar axis. East and West ansa readings did not differ significantly and were averaged in the final result. A check of scattered disk radiation at a polar sky position 18 arc s from the disk centre gave no measurable reading.

The ratio of the signals obtained for the ring and disk positions was 0.39 ± 0.05 in the $34 \mu\text{m}$ band and 0.49 ± 0.02

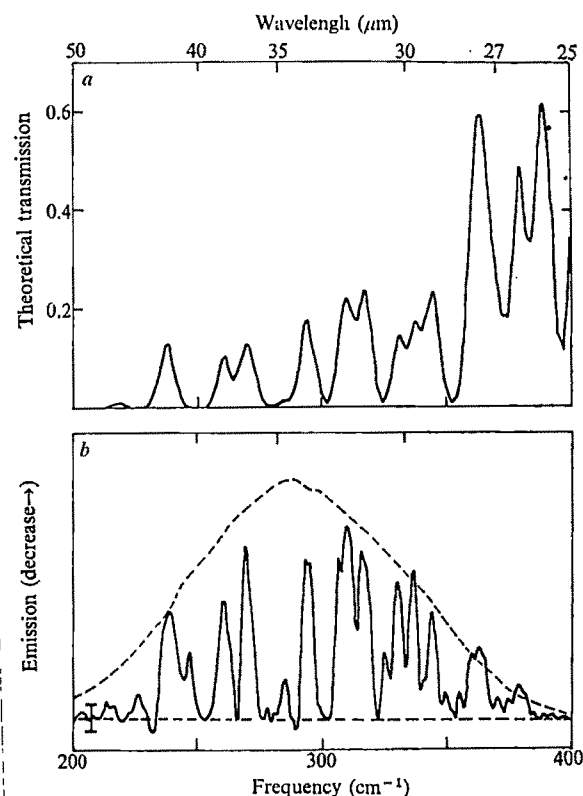


FIG. 1 Measured sky emission, compared with theoretical atmosphere transmission. *b*, Sky emission spectrum ($\sim 2 \text{ cm}^{-1}$ resolution) measured with the $34\text{-}\mu\text{m}$ filter and detector system attached to an interferometer. The saturated portions of the sky emission spectrum were used to establish the dashed baseline. The dashed line spectrum is computed from laboratory blackbody measurements, at a lower resolution, and shows the emission which would be observed on the same scale from a 270 K blackbody with an emissivity of 0.2 and the same filter system. *a*, Corresponding spectral features in a theoretical atmosphere transmission spectrum which has been computed by V. Kunde for 0.5 mm of atmospheric water content and a spectral resolution of 5 cm^{-1} .

in the 20 μm band, where the errors represent one standard deviation of the mean. To correct the 34- μm measurement for instrumental effects, we compared the 20- μm result to the transmission-weighted spectral ratio for the rings and disk of 0.82 ± 0.02 determined from Murphy's 20- μm band brightness temperatures⁶. We assumed 1 mm of precipitable water vapour for determining the transmission-weighting function. This value for the 20- μm observations was inferred from daytime water vapour observations. The difference between the present ratio 0.49 and 0.82 from Murphy's data gives the composite correction for aperture filling factors (geometrically ~ 0.7 for the ring position), diffraction of ring flux out of the aperture and different source 'dwell times' with our sinusoidal chopping for the ring and disk readings. From this 20- μm calibration procedure, we deduce that the transmission-weighted specific intensity ratio of the rings (A and B) to the disk is 0.67 ± 0.13 in the 34- μm band. This allows for an additional 3% diffraction loss computed for the ring signal at this longer wavelength. We have assumed that systematic errors due to centering differences are small.

The interpretation of our results does not depend on knowing the absolute transmission and depends on the relative transmission spectrum only in so far as the ring and disk spectra differ. Because of the collision-induced H_2 opacity source at 28 μm , model atmosphere calculations depart from that of a blackbody in this spectral region⁹. Figure 1 compares a zenith sky emission spectrum, obtained during dry atmospheric conditions in March, with the theoretical transmission for 0.5 mm precipitable water vapour content. We divided the sky emission spectrum of Fig. 1b, which also incorporates the filter transmission properties, by a 270 K Planck spectrum. This result approximates the relative atmospheric-system transmission for weighting model spectra of the rings and disk. Saturn was observed at 34 μm through less than 1.2 air masses and with atmospheric conditions comparable to those for the spectrum shown in Fig. 1b.

We can use the 34- μm observations to derive a model effective temperature for the planet by assuming: (i) that the A and B rings exhibit the same average brightness temperature of 91 ± 3 K in the 34- μm band as in the 20- μm band⁶; and (ii) that Trafton's model atmosphere calculations represent the planetary spectrum⁹. We obtain a model effective temperature of 103 ± 6 K by an extrapolation of Trafton's spectrum for a 100 K model with zero helium abundance. This result compares to a blackbody brightness temperature of 99 ± 6 K for the planet in our 34- μm bandpass. Present models of the ring involving large particles lend some support to an assumption of a fairly constant ring brightness temperature between 17 and 43 μm (ref. 10). The lower ring temperature of 83 K observed at 12 μm in 1969 (ref. 4) can be explained by assuming the ring brightness temperature increases as the Saturnicentric declination of the sun increases⁶.

We can also use aircraft measurements of the total system flux to estimate ring and disk temperatures. Armstrong *et al.*¹ report a composite brightness temperature of 89 ± 4 K in the band 30 to 45 μm . Their quoted error does not allow for the uncertainty in absolute flux calibration. The resolution of this measurement into ring and disk components, consistent with our measurement of their relative emission in approximately the same bandpass, yields an average blackbody brightness temperature of ~ 86 K for the rings and a model effective temperature of ~ 96 K for the planet. These temperatures do not differ significantly from those derived above if we allow for the absolute flux calibration uncertainty in the aircraft measurement and differences in bandpass characteristics.

The results reported here support previous suggestions of a large internal heat source for Saturn². For example, using Walker's bolometric albedo¹¹ of 0.61, we calculate a solar equilibrium temperature of 71 K. A model effective temperature

of 96 to 103 K implies an internal heat source of 2.3 to 3.4 times the solar insolation.

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Reflection from a transversely moving mirror

ALTHOUGH there have been many experiments which demonstrate the first order Doppler effect in the reflection of electromagnetic waves from a mirror with a component of radial motion, there has not yet been a precise determination of the frequency of light reflected from a transversely moving mirror. There is some confusion about whether light reflected from a mirror moving transversely to an incident beam should be reflected at a frequency which is different from that of the beam, because of a relativistic (second order Doppler) effect^{1,2}. Any frequency shift would be extremely small, and there are severe experimental difficulties in attempting to observe a shift of magnitude equal to or less than that associated with the transverse Doppler effect. The experiment described here was designed to overcome these difficulties and to investigate the possibility of frequency shifts of a smaller magnitude than that which would correspond to the second order Doppler shifts associated with transversely moving emitters³. The overall accuracy of the experiment is better than one part in 10^{16} .

A rotating mirror, supported by an air bearing, is in one arm of a Michelson interferometer. The other arm has a

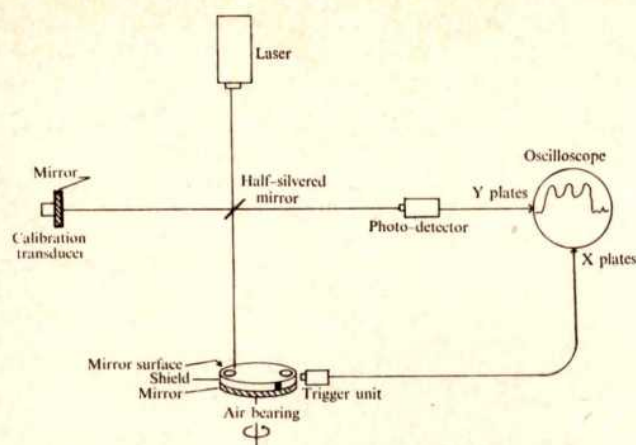


FIG. 1 The arrangement for synchronising the display from a Michelson interferometer in order to investigate any frequency shift in light reflected from a transversely moving surface.

mirror which can be moved in the direction of the reference light beam by a crystal transducer. This mirror, when moved, demonstrates that the equipment is functioning correctly, by changing the position of the fringes displayed on the oscilloscope screen.

The fringes are monitored by the photodetector, which delivers an output to the 'Y' plates of an oscilloscope. The oscilloscope time-base is triggered at the same frequency as the rotation of the mirror. Thus each particular point on the mirror corresponds to one particular part along the 'X' axis of the display. This removes the effects of variations in the mirror profile and of periodic vibrations of the rotating mirror which are related to the frequency of rotation.

Any relativistic frequency shift produced by the transverse motion should cause the two beams to beat together and thus the pattern displayed on the oscilloscope should undulate at each value of X. The control mirror, which may be considered as introducing a first order Doppler effect, shows this effect quite clearly. Calculations show that at the transverse speeds of $5\text{--}20\text{ m s}^{-1}$ used in the experiment, beat frequencies of the order of one beat per second to one beat in twelve seconds would be produced by a mechanism of the magnitude of the second order Doppler effect for a transversely moving emitter. Observation for 10 times the associated beat period showed no evidence of any shift in the position of the fringes in the display. The apparatus is being modified and we hope to improve the accuracy of the experiment and then to publish the results in greater detail.

An explanation of why no frequency shift occurs has been produced by using standard transforms of direction cosines of electromagnetic waves. The light is received at the mirror surface at an angle of approach and shifted to the blue. The blue light is then emitted specularly at the mirror, making an equal and opposite angle to the normal to the mirror surface. This reflected beam is received back in the laboratory at normal incidence and restored once more to its original colour. Einstein's equation⁴ is a restricted analysis of the complete phenomenon. It is restricted to motions in the direction of the normal to the mirror surface. Reflection by a transversely moving mirror is the other special case.

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Discovery of three earthquake faults in Iran

An earthquake fault is a ground fracture formed in association with a shallow earthquake, usually by reactivation of a pre-existing geological fault. It can provide information about both the mechanism of seismic energy release during an earthquake and the accompanying regional strains. Until now only two such faults which have been adequately documented are known in Iran. These are associated with the Buyin Zara (1962) and the Dasht-e Bayaz (1968) earthquakes¹⁻³. The Torud (1953) and the Ashkhabad (1948) earthquake^{4,5} (the latter in the USSR, just north of the Iranian frontier), were probably also accompanied by faulting, but complete documentation is lacking. Here, we describe three additional earthquake faults found during recent seismotectonic field studies in Iran. The earthquakes involved were at Selakhor (1909), Baghan-Germab (1929) and Salmas (1930). Figure 1 shows the location of the three faults and of the other known and probable faults already mentioned.

The Selakhor earthquake occurred at 02 h 48 min 18 s GMT on January 23, 1909 in the upper Ab-i Diz valley in the Selakhor District of the Zagros mountains (south-western Iran). The body wave magnitude of the event was 7.4 (ref. 6). Maximum destruction was confined to the south-eastern part of the valley, between the village of Zargina and the small town of Dorud, with the preliminary macroseismic epicentre (taken as the centre of the area of maximum destruction) located at about $33.5^{\circ}\text{N } 49.0^{\circ}\text{E}$ (Fig. 2).

Contemporary descriptions of ground deformation⁷ suggest that faulting occurred during the earthquake. The eroded fault scarp is clearly visible now on the ground and on aerial photographs, and can be followed in a 135° direction from Kalangona to Dorud and Sarawand (Fig. 2). In the alluvium of the Ab-i Diz Valley it appears as a rectilinear



FIG. 1 Location of known earthquake faults in Iran. Dates indicate year of earthquake associated with faulting, with double frame for the three faults studied here. Question mark for cases in which documentation is incomplete.

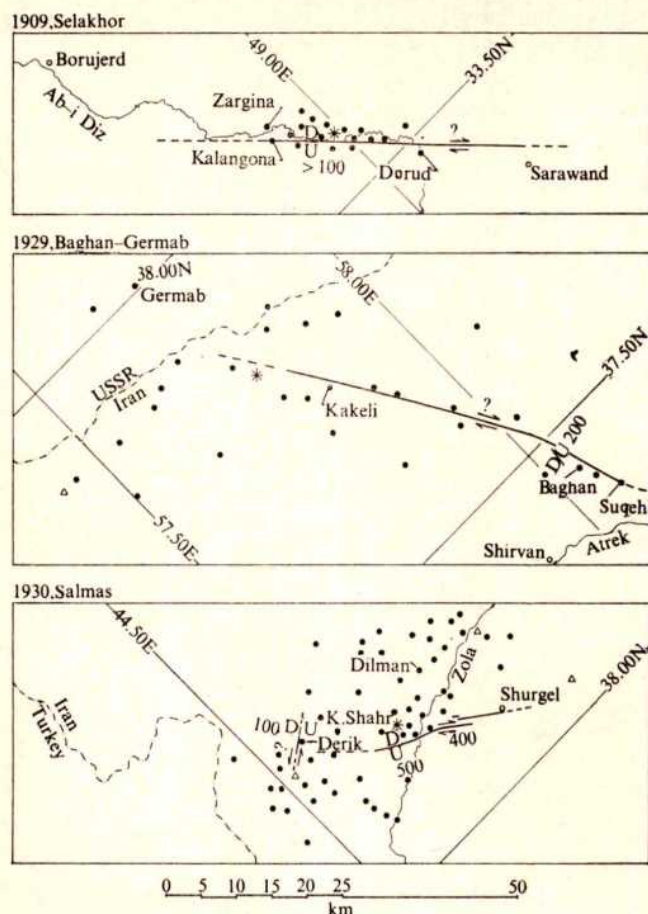


FIG. 2 Earthquake fault trace and damage distribution. Black line indicates fault trace, dashed where uncertain. Vertical fault displacements during the earthquake are shown by U (up) and D (down) and horizontal displacements by double arrow. Numbers are displacements in cm, with question mark where sense of displacement is known but not the amount. ●, Villages totally destroyed or very severely damaged. *, Approximate centre of area of maximum destruction, taken as macroseismic epicentre. △, New thermal spring created, or existing spring modified, by the earthquake.

topographical step along which the north-eastern side is downthrown by at least 1 m. Horizontal displacements have so far not been determined. South-east of Dorud, the earthquake fault enters the mountain range where it can be seen to coincide with a well marked pre-existing geological fault. The total length of the 1909 fault scarp exceeds 40 km.

Detailed geological mapping has shown that the earthquake fault was formed by reactivation of a much larger NW-SE structure, the Main Recent Fault. This is a major wrench fault extending over 1,700 km from eastern Anatolia to the Gulf of Oman⁸. It is later than, and quite distinct from, the post-Pliocene Main Thrust^{9,10} which is considered to mark the northeastern edge of the Arabian Plate¹¹ (Fig. 1). The Main Recent Fault probably results from the latest NNE motion of the Arabian Plate with respect to Central Iran, and is at present seismic to the north-west of the 1909 epicentral region, but in the south-east no data has yet been found to establish its activity.

The Baghan-Germab earthquake occurred at 15 h 37 min 36 s GMT on May 1, 1929 in the Kopet Dag mountains (north-eastern Iran) with a magnitude of 7.1 (ref. 6). Maximum destruction and ground deformation were observed along a NNW-SSE zone between Baghan (Iran) and Germab (USSR), with the preliminary macroseismic epicentre located at 37.8°N 57.8°E (Fig. 2).

Surface faulting over a length of about 50 km was associated with the earthquake, extending across the fold

trend in a 150° direction from Suqeh to the vicinity of the Soviet border (Fig. 2). It was mapped along two sections, at Baghan and at Kakeli, and the overall trace was drawn on the basis of aerial photographs together with information from contemporary accounts¹² and interviews with survivors of the catastrophe. Today, the eroded scarp is in places up to 2 m high, with the north-eastern side uplifted (Baghan region). There is also some indication of dextral strike-slip movement, but more field work is required to obtain accurate measurements of this displacement. The linearity of the earthquake fault trace through varying topography indicates that it is probably vertical at depth.

The 1929 earthquake fault resulted from reactivation of a post-Pliocene fault zone which extends from Bakharden (USSR) to Quchan (Iran) (Fig. 1). This zone forms part of a fault system which dissects the late Alpine folds of the Kopet Dag and consists of NNW dextral and NE sinistral strike-slip faults and minor E-W thrusts. This system is consistent with a NNE-SSW direction of tectonic compression. Quaternary horizontal displacements on some of the individual faults amount to several kilometres¹³, and several have been shown to be active¹⁴. In the north, the Bakharden-Quchan fault zone bends into the Main (Kopet Dag) Fault which marks the southern edge of the Turan Plate. In the south the fault zone crosses the Atrak River near Quchan, which has been devastated at least three times by earthquakes during the 19th century.

The Salmas earthquake occurred in west Azarbaijan (north-western Iran) on May 6, 1930, almost exactly one year after the Baghan-Germab earthquake. It was preceded on the same morning by a moderately strong foreshock. The main shock, which occurred at 22 h 34 min 27 s GMT, had a magnitude of 7.2 (ref. 6) and caused extensive destruction throughout the Salmas valley and the mountains in the south and west near the Turkish frontier (Fig. 2). The preliminary macroseismic epicentre was located at about 38.2°N 44.7°E.

The earthquake fault scarp can be seen over about 20 km between Shurgel and the region west of Kohneh Shahr at the southern edge of the Salmas valley (Fig. 2). Its average direction is 120° and maximum displacements during the earthquake were about 4 m dextral and 5 m



FIG. 3 Salmas earthquake, 1930: Fault scarp south of Kohneh Shahr. The scarp crosses an early Christian cemetery (from bottom right of photograph to background). The ground, which was originally level, dropped by a maximum of 5 m north of the fault (left of photo). The two figures at foot of scarp provide a scale.

downwards on the north-eastern side (Fig. 3). Survivors of the earthquake describe the fault as extending another 10 km to the north-west, but no trace of this scarp can now be seen. At Derik, a separate earthquake fault downthrown about 1 m to the north-west extends for about 3 km through metamorphic rock in a 55° direction. The pattern formed by the location of Quaternary and active travertine springs suggests that the corresponding geological fault has a sinistral horizontal component. At the south-western end of the Derik fault a thermal spring ceased at the moment of the earthquake, and a new one appeared about 1 km further south-west in the same alignment. Other thermal springs appeared, and an existing one at the south-eastern end of the main fault segment increased its flow.

The sense of vertical displacement along the main earthquake fault, and observations of waterlogging in the fields of the Salmas valley, show that the valley subsided during the earthquake. The importance of the dextral displacements, however, and the movement along the Derik fault, indicate a more complex tectonic situation which may be interpreted in terms of a N-S or a NNE-SSW compression. The structure of this part of the country, which is now being mapped by the Geological Survey of Iran, has not yet been studied and it can only be noted at this stage that the general NW direction of the earthquake fault is also that of the major regional active faults: one passing through Tabriz and Maku in the north, the Main Recent Fault (Zagros) in the south, and the North Anatolian Fault further west, in Turkey.

The small number of earthquake faults known in Iran is surprising in view of the high seismicity of the country and the presence of faults known to be active¹⁵. This is possibly because aseismic fault activity (fault creep) plays a large part in the process of tectonic strain release. There is also a lack of adequate macroseismic documentation. In this respect it is interesting to note that four out of the five earthquake faults known with certainty (Fig. 1) were over 40 km long, and that all were associated with events of magnitude greater than seven. It seems probable that, as in other seismic countries^{16,17}, shorter surface breaks have occurred in Iran during earthquakes of smaller magnitudes, but that these earthquake faults have passed unreported or even, in desert areas, unnoticed. The three earthquakes summarised here emphasise the danger, common in Middle Eastern seismotectonic research, of drawing conclusions on the only available basis of instrumental epicentre data covering the last one or two decades. With such data the regions of the 1909, 1929 and 1930 earthquakes seem to be relatively quiescent, and the activity of the associated faults is not noticed.

It would be premature at this stage to integrate the new information presented here with existing knowledge on the tectonics of Iran. The common characteristic of the three earthquake faults should, however, be kept in mind during future field studies. In all three cases, fault directions and displacements are most easily interpreted as indicating present day NNE-SSW compression. This manifests itself in the regions of contact between Central Iran and the adjoining plates—the Arabian Plate in the south-west (1909 earthquake) and the Turan Plate in the north-east (1929 earthquake), as well as in the slightly more internal region of West Azarbaijan (1930 earthquake). A predominantly NNE direction of plate motion has been postulated for Arabia on the basis of fault plane solutions for recent earthquakes¹⁸, but field evidence is so far lacking. It remains to be seen whether shallow earthquakes in more central parts of Iran may be similarly interpreted.

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Geology and volcanic history of Pico Island Volcano, Azores

THE Azores Islands formed as a result of volcanism along a conservative plate margin¹. The volcanism and seismicity of the archipelago have been described² together with chemical analyses of lavas from many of the islands³.

The Mid-Atlantic ridge crest isolates the most western islands, Flores and Corvo, from the rest of the archipelago. The semismicity of the ridge branches eastwards along a more subdued ridge; the Azores-Gibraltar-Rise. The latter is primarily a strike-slip fault zone with tensional characteristics and associated volcanism in the Azores region, and a compressional zone in the region immediately west of the Straits of Gibraltar^{4,5,6}.

A secondary spreading ridge has formed in the Azores region, resulting from a combination of a different spreading rate north and south of the archipelago and a change in crustal spreading direction⁷. The secondary spreading centre is a graben known as the Terceira Rift⁸, within which three of the major Azorean volcanoes (Graciosa, Terceira and S. Miguel) have formed.

The earliest volcanism occurred in the extreme east of the island group, where basalt lava flows are exposed beneath limestones palaeontologically dated as Middle Miocene⁹. Potassium-argon dates have not yielded ages greater than 4 m.y. for lavas on Santa Maria Island and on the

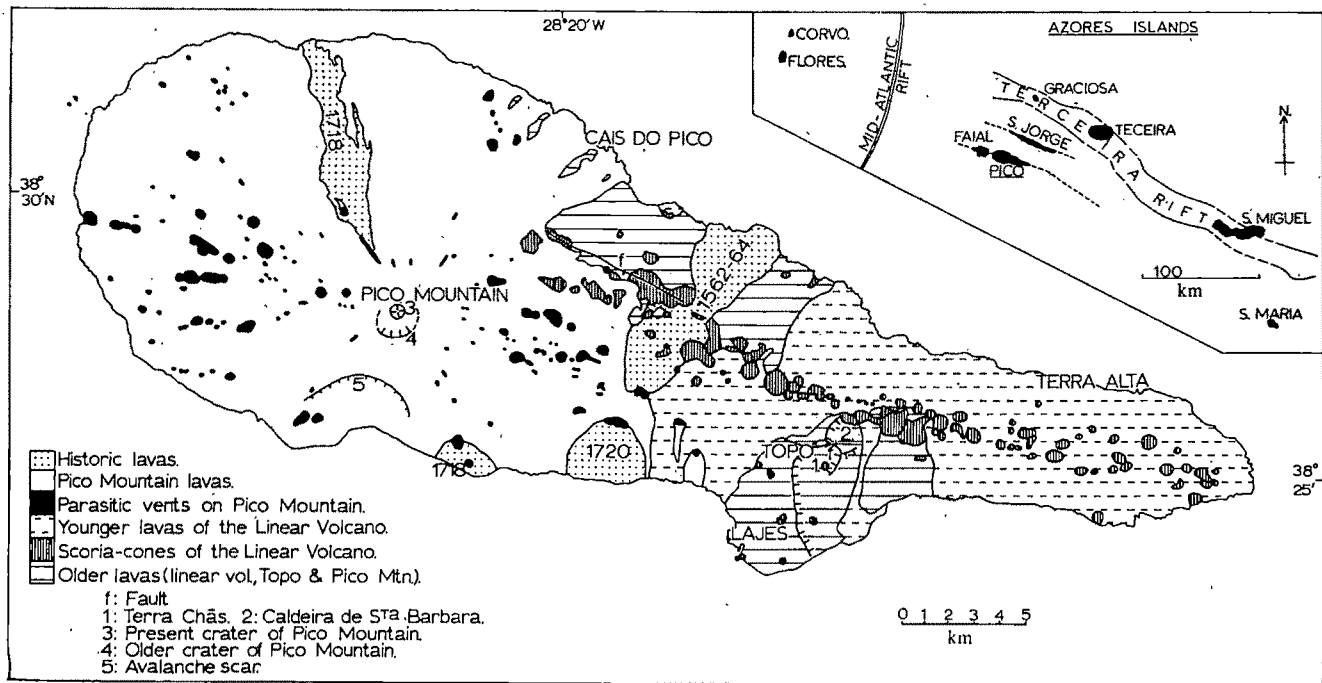


FIG. 1 Geological map of Pico Island Volcano.

extreme east of Sao Miguel^{10,11}. The latest volcanism occurred in the islands of the central group, of which Pico is a member.

Pico Island seems to be the youngest island in the Azores and is covered by many recent basaltic lava flows. Eruptions were recorded in 1562–64 and 1718–20. The island has been mapped by the Portuguese Geological Survey¹².

There is a central volcano, Pico Mountain (2,351 m), in the west and a linear volcano (1,076 m) dominates the eastern region (Fig. 1). The island is 46 km long and has a maximum width of 16 km. The volcanoes rise 3,500 m from the sea-floor and lie on a linear submarine ridge trending parallel to the Terceira Rift (Fig. 1, inset), which also includes the adjacent island of Faial.

The flanks of Pico Mountain are covered with recent lava flows erupted from radially aligned parasitic fissures. The latest activity comprises flank eruptions rather than eruptions from the central cone. The cone rises steeply above an altitude of 1,200 m with slopes up to 40°. Below this altitude, however, the slopes are seldom steeper than 8–10°. Probable gravity sliding on the steep flanks of the mountain has created a large avalanche scar (Fig. 1) on the south slopes. The slopes of Pico Mountain are composed of pahoehoe lavas derived directly from the summit crater (500 m diameter), which has since become filled almost completely by similar lavas. Fumaroles with temperatures of up to 60° C occur on the flanks of a lava cone which has formed in the north-eastern region of the crater.

The construction of the steep sided cone of Pico Mountain has probably resulted from repeated eruption, at very low rates of effusion, of pahoehoe lavas with little associated tephra, giving rise to highly compound lavas such as those exposed in the summit crater and at the foot of the mountain¹³. The flank lavas include both pahoehoe and aa flows.

The lavas of Pico Mountain contain phenocrysts of olvine and augite, in some cases with plagioclase. Crystal settling has occurred in many pahoehoe flows of ankaramiti nature, involving the accumulation of large olivine and augite phenocrysts in the lower part of a flow. Radiating plagioclase glomero-groups characterise the pahoehoe lavas of the steep sided cone. The historic Santa Luzia flow (1718), north-west of the mountain, (Fig. 1) also contains phenocrysts of hornblende, magnetite and apatite.

The Linear Volcano is offset to the north relative to Pico

Mountain. It has evolved by repeated fissure eruptions along WNW trending fissures. Young lava flows occur along the entire 30 km length, except in the extreme north-west.

The Topo Volcano is situated halfway along the Linear Volcano, slightly to the south of its main axis (Fig. 1). It is controlled by NE–SW trending fissures. Evidence suggests, however, that it has evolved as a volcano transitional between a linear and a central volcano. It is surrounded by the younger lavas of the Linear Volcano and it conveniently divides the latter into a western and an eastern Linear Volcano. No young lavas occur on the Topo Volcano.

The western Linear Volcano is divided equally into an older north-western region characterised by eroded scoria-cones together with recent fault scarps, and an eastern region dominated by young scoria-cones situated along a narrow fissure zone (Fig. 1).

The eastern Linear Volcano is also dominated by young scoria-cones, but older cones, partially buried by the younger lava flows, also occur. High cliffs which are mostly degraded,

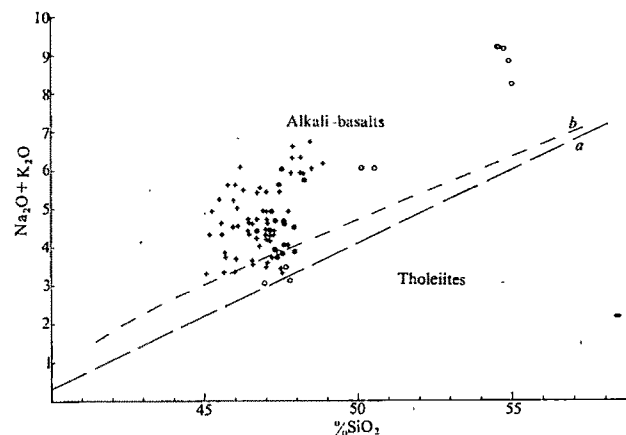


FIG. 2 Alkalies: silica diagram for Pico rocks: ●, lavas of Pico Mountain central volcano; ○, lavas of 1718 and 1720 (Pico Mountain volcano); +, lavas of the Linear Volcano and Topo. Dashed lines divide alkali basalts from tholeiites: a, from Macdonald (1968); b, from Irvine and Baragar (1971).

such as Terra Alta, occur on both the north and south sides of this narrowest zone of the island (Fig. 1).

The younger lavas of the Linear Volcano seem to be penecontemporaneous with those of Pico Mountain.

The Topo Volcano had an old summit crater which subsequently became filled in by thick pahoehoe lavas. These may have formed a lava lake. The original crater region has been modified by caldera collapse of the 'trap door' type¹⁴ with arcuate scraps only partially enclosing a caldera, for example, the Terras Chas Depression and the Caldeira de Santa Barbara (Fig. 1). Parasitic vents and fault scraps occur to the south-west of the collapse region, and thin dykes together with some buried scoria-cones are exposed in the cliffs along the south coast.

Early in its history the Topo Volcano probably resembled the shield volcanoes of the western Galapagos Islands¹⁵ but the morphology has been modified by the caldera collapse. Only four lavas seem to postdate this event. The oldest lavas of the adjacent Linear Volcano postdate all but the latest Topo lavas.

The oldest lavas on Pico occur in the north-western region of the Linear Volcano, and on the Topo Volcano, and in both cases the lava flows are covered by ash and/or soil. Scoria-cones are considerably eroded. The oldest lavas are exposed at the foot of the high cliffs along the south coast of the Topo Volcano. Similarities between the two areas suggest that they are probably of the same age.

The lavas of the Linear Volcano are dominantly aa flows. There are very few pahoehoe lavas, although an extensive flow was erupted in the period 1562-64, from the older, western region (Fig. 1). The majority of the lavas contain phenocrysts of olivine, augite and plagioclase but older lavas may contain only olivine phenocrysts. Many of the Topo lavas are similar to those of Pico Mountain, and spectacular crystal settling, involving the accumulation of olivine and augite phenocrysts, characterises many of the pahoehoe lavas. This phenomenon is also present in the thick pahoehoe lavas which fill the old, pre-caldera, summit crater referred to already. Less porphyritic aa flows are common together with aphyric lava flows which are more commonly associated with the Topo Volcano. There are also plagioclase rich basalts which resemble those on the steep sided cone of Pico Mountain. Biotite occurs as a rare accessory mineral in some Topo lavas.

Figure 2 indicates the alkaline nature of the analysed lavas of Pico. Some hypersthene normative lavas occur and they plot on the boundary between tholeiites and alkali basalts^{16,17}. The most highly differentiated lava is a single flow of benmoreite erupted on the northwestern flanks of Pico Mountain in 1718. This is part of a lava series erupted in 1718 and 1720, which ranges in composition from olivine basalt (hypersthene normative) to hawaiiite and then to benmoreite.

Few differentiated lavas occur on Pico Mountain, and there is only very slight differentiation in the older lavas of the Linear Volcano and Topo. The younger lavas (Fig. 1) of the Linear Volcano are interpreted as a differentiated series akin to that mantling the older 'oceanite' series on Reunion Island (Indian Ocean)¹⁸. The Pico differentiated series ranges from plagioclase basalts to hawaiiite and includes two flows transitional to mugearite.

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Heteromolecular clusters of H₂O, SO₂, CO₂, CO and NO

HOMOGENEOUS clusters of gas molecules in isentropically expanding jets, first observed in 1961^{1,2}, have been the subject of several experimental studies³⁻⁷. Mixed or heteromolecular clusters have also been observed in mixtures of two or more gases expanded from a high pressure reservoir. Leckenby *et al.*⁸ have identified the species Ar·CO₂, Ar·(CO₂)₂ and larger clusters, and N₂·O₂, SF₆·(CO₂)₂ and SF₆·CO₂·Ar. Milne *et al.*⁹ have observed mixed H₂O-Ar clusters and the heteromolecular dimers O₂·H₂O and NO·H₂O.

We observed mixed clusters of the type SO₂·(H₂O)_n, *n* = 1-6, of the same order of magnitude as pure water clusters when 2 mmHg of SO₂ were added to 1,500 mmHg nitrogen, saturated with water of room temperature, and expanded through an orifice of 0.0386 cm. The possible implications of the homogeneous formation of such hydrated cluster species to upper atmospheric chemistry¹⁰ and aerosol formation¹¹, led to a series of experiments with a water-saturated nitrogen carrier which was seeded with trace gas species

TABLE 1 Heteromolecular clusters observed in fully expanded jets*

Species	<i>n</i>	Concentration in nitrogen
SO ₂ (H ₂ O) _n	1-5	0.5% SO ₂
CO ₂ (H ₂ O) _n	1-5	5% CO ₂
NO(H ₂ O) _n	1-6	1% NO
H ₂ O(SO ₂) _n	1,2	0.5% SO ₂
(CO ₂) ₂ (H ₂ O) _n	1,2	5% CO ₂
(NO) ₂ H ₂ O	-	0.5% NO, 0.25% SO ₂
NO·SO ₂ ·(H ₂ O) _n	1-3	0.5% NO, 0.25% SO ₂
CO ₂ ·SO ₂ ·H ₂ O	-	2.5% CO ₂ , 0.25% SO ₂
NO(SO ₂) _n	1,2	0.5% NO, 0.15% SO ₂
NO(CO ₂) _n	1,2	0.5% NO, 2.5% CO ₂
CO ₂ (NO) _n	1,2	2.5% CO ₂ , 0.5% NO
SO ₂ (CO ₂) _n	1,2	0.25% SO ₂ , 2.5% CO ₂
CO(CO ₂) _n	1-3	0.5% CO, 2.5% CO ₂ †

* Total source pressure, *P*₀ = 2,000 mmHg. Orifice diameter, *d* = 0.0343 cm. The first eight experiments were carried out with nitrogen saturated with water of room temperature. The remainder were conducted with dry nitrogen. The species N₂·H₂O, N₂·SO₂, N₂·(SO₂)₂, N₂·CO₂, and N N₂·NO were also observed mainly as a result of the large amount of nitrogen present.

† Under the present conditions CO did not cluster appreciably with any species except CO₂.

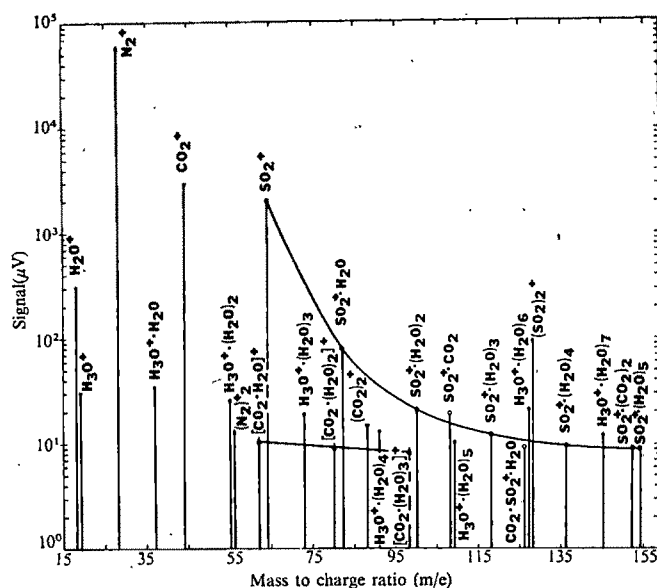


Fig. 1 Heteromolecular clusters observed in a fully expanded jet composed of 10% H_2O , 2.5% CO_2 and 0.25% SO_2 in nitrogen. The $\text{SO}_2^+(\text{H}_2\text{O})_n$ and the $[\text{CO}_2(\text{H}_2\text{O})_n]^+$ series are connected by curves for convenience.

which are known to exist in the atmosphere and in the exhausts of jet aircraft.

The supersonic molecular beam apparatus consists essentially of a three-stage differentially and cryogenically pumped vacuum system^{5,6}. Gases and/or vapours are admitted through a sonic orifice at the end of a source probe, and the beam is formed by a helium-cooled skimmer (20° K) and then further collimated. Beam species were detected with a quadrupole mass spectrometer after mechanical beam chopping and subsequent phase-sensitive signal processing with a lock-in amplifier. For some of the latest experiments the mass spectrometer was controlled by a minicomputer and the ion signals were digitised.

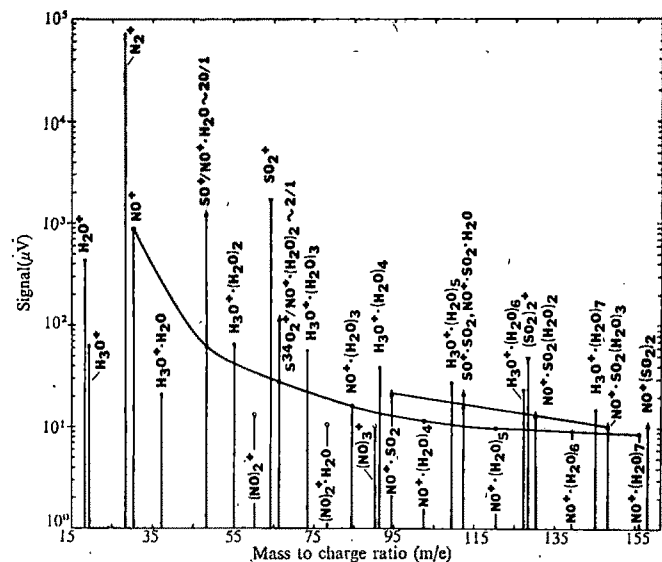
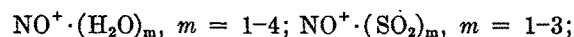


Fig. 2 Heteromolecular clusters observed in a fully expanded jet composed of 10% H_2O , 0.5% NO and 0.25% SO_2 in nitrogen. The $\text{NO}^+(\text{H}_2\text{O})_n$ and the $\text{NO}^+\text{SO}_2(\text{H}_2\text{O})_n$ series are connected by curves for convenience. The $\text{SO}_2^+(\text{H}_2\text{O})_n$ series was also present in this expansion but it is not shown here in order to simplify the spectrum. It is of approximately the same magnitude as in Fig. 1.

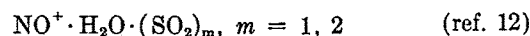
Table 1 summarises the experimental conditions and the mixed cluster species observed. Figures 1 and 2 present

sample mass spectra to give an indication of the relative magnitudes of the ion signals. The total source pressure and orifice diameter for both cases are 2,000 mmHg and 0.0343 cm respectively. Only those peaks directly related to the heteromolecular clusters are shown, with the water cluster ion series (that is, $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$) included for comparison. The ionising electron energy was 90 V. The species actually detected were ions produced in the mass spectrometer ion source. The ionisation occurred, however, in the free molecular flow regime after all collisions had ceased and, therefore, the formation of the heteromolecular clusters was completely homogeneous. Ion-molecule reactions can be excluded because of the low pressure in the mass spectrometer ion source (about 10^{-8} mmHg) and because the observed signals were in phase with the beam chopper. A more extensive analysis of the results will be published elsewhere.

Species similar to those in Table 1 have been heterogeneously generated by subjecting low pressure gas mixtures (1–4 mmHg) to an electrical discharge and allowing them to react in a field-free reaction cell¹². In this manner the following cluster ions were observed mass spectrometrically:



and



Pending further investigation, we think that the observed mixed cluster signals are mostly virgin species formed through a simple gas kinetic mechanism, rather than from complex fragmentation of a condensed bulk phase on electron impact in the ion source of the mass spectrometer. Although the low temperatures attained in the nitrogen expansion are certainly sufficient to condense water, thus raising the possibility of some fragmentation of the bulk phase, there is some experimental¹³ and theoretical¹⁴ evidence to suggest that this is secondary to other effects. Also, in a recent experimental CO_2 condensation study, massive fragmentation was presumed absent because of the agreement between the observed and predicted dependence of cluster concentrations on source pressure and orifice diameter¹⁵.

The formation of the heteromolecular dimer probably is the rate-controlling step in the growth of mixed clusters. The dimer must be formed by a termolecular collision and higher clusters then grow by the successive bimolecular addition of monomers. A simple gas kinetic model with termolecular dimer formation and bimolecular destruction seems to be adequate to predict dimer concentrations in expanding jets^{5,15}. The surprising persistence of the species (Table 1), despite the relatively low concentrations of the apparent species in nitrogen, can only be explained by postulating strong affinity, with nitrogen serving as the third body in the dimer formation mechanism.

The results presented here have established the existence and identity of mixed clusters of H_2O , SO_2 , CO_2 , NO and CO formed during homogeneous nucleation processes in an isentropic expansion from the gas phase. There are numerous observations of the homogeneous nucleation of water vapour which has been appreciably enhanced by the presence of foreign gas molecules¹⁶⁻¹⁸. Clathrate structures of water molecules around foreign gas molecules, particularly those found as trace contaminants in the atmosphere, and most notably, in the present context, SO_2 and CO_2 may be important in aerosol physics, and furthermore, favourable conditions for the production of water clathrates are known to be present in the exhausts of jet aircraft⁹.

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Non-linearity of hydrogen bonds in molecular crystals

In recent studies of the non-linearity of hydrogen bonds^{1,2}, histograms have been shown of the O-H ... O angles, ψ . The distributions reflect the energy dependence of the hydro-

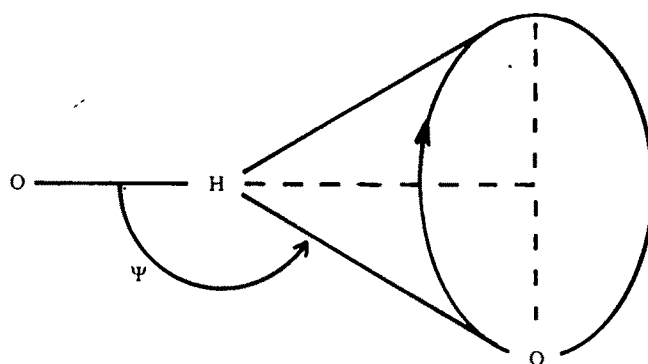


FIG. 1 Cone of opening angle ($360^\circ - 2\psi$) illustrating the number of possible hydrogen-bond configurations with O-H ... O angle, ψ .

gen bond on the angle ψ . In such distributions maxima are found at values of ψ approximately 15° from the linear configuration. Model calculations on water dimer interactions³ indicate, however, that the configuration of minimum energy involves a linear hydrogen bond.

Before angle distributions of the hydrogen bond as observed in crystal structures, are compared with results of theoretical calculations on non-linearity in simple hydrogen bond models, experimentally determined hydrogen bond angle frequencies should be properly adjusted. This is because the sampling procedure includes a factor which complicates the correct interpretation of the histograms. This factor, which is of a geometric nature, seriously influences the statistics and has not been taken into account in other studies. This factor does not affect the observed hydrogen bond angle in any single case, but only affects the frequency distribution of the whole set of bond angles.

Assuming that the optimum hydrogen bond is linear this factor depends on ψ , because the number of possible hydrogen bond configurations at any value of ψ is proportional to $\sin \psi$. This is shown in Fig. 1, which illustrates that the acceptor oxygen atom at a certain O-H ... O angle, ψ , can select any of the positions that lie on the generator of a cone of opening angle $360^\circ - 2\psi$, with the hydrogen atom at the apex and the cone axis in line with the O-H bond.

Thus although the assumption that the energy depends on ψ indicates that the probability of a hydrogen bond with angle ψ decreases as ψ increases, in fact the probability will increase with increasing ψ because of the increase in the number of possible hydrogen bond configurations with angle ψ .

TABLE 1 Crystal structures selected for the hydrogen bond study

Compound	Ref.	Compound	Ref.
Allitol	4	γ -D-gulonolactone	27
Methyl α -D-altropyranoside	5	D-iditol	28
D-L-arabinitol	6	Inosine	29
β -D-L-arabinose	7	Epi-inositol	30
L-ascorbic acid	8	Myo-inositol	31
Dehydro-L-ascorbic acid	9	1-kestose	32
D-iso-ascorbic acid	10	α -lactose. H ₂ O	33
Cellobiose	11	β -lyxose	34
Methyl β -cellobioside-methanol	12	Methyl β -maltopyranoside. H ₂ O	35
2, 6-anhydro- β -D-fructofuranose	13	Isomaltulose. H ₂ O	36
Galactitol	14	D-mannitol (K Form)	37
D-galactono- γ -lactone	15	β -D-mannitol	38
Methyl α -D-galactopyranoside. H ₂ O	16	Methyl α -D-mannopyranoside	39
D-glucitol (A form)	17	Planteose. 2H ₂ O	40
Glucitol-pyridine	18	Raffinose. 5H ₂ O	41
D-glucono-(1, 5)-lactone	19	α -rhamnose. H ₂ O	42
1, 6-anhydro- β -D-glucopyranose	20	Ribitol	43
α -D-glucose. H ₂ O	21	α -L-sorbose	44
β -D-glucose	22	Sucrose	45
α -D-glucose-urea	23	α , α -trehalose. 2H ₂ O	46
1, 6: 2, 3-dianhydro- β -D-gulopyranose	24	Xylitol	47
Methyl α -D-glucopyranoside	25	α -xylose	48
β -D-glucurono- γ -lactone	26		

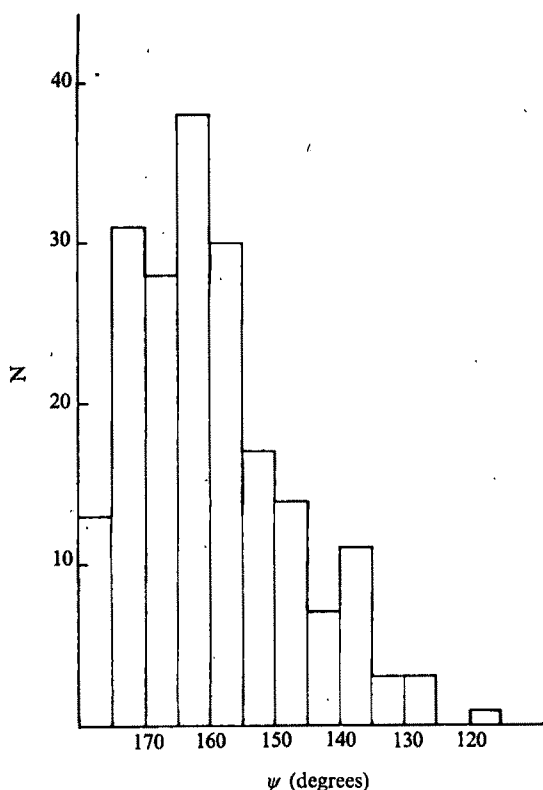


Fig. 2 Histogram of the 196 O-H...O hydrogen bond angles observed in the crystal structures listed in Table 1.

As a result of this, the maximum in the frequency distribution may be expected to occur appreciably away from 180°. In other words, conclusions about the hydrogen bond energy from angle frequency distributions can only be compared with theoretical model calculations if the spatial freedom factor is either included in the theoretical model calculations or removed from the experimental hydrogen bond angle frequency distribution.

We applied the $\sin \psi$ correction to the statistics of a series of 196 hydrogen bond angles collected from recent literature⁴⁻⁴⁸. Only one type of hydrogen bond was considered: the donor and acceptor groups were aliphatic hydroxyl groups in polyalcohols, saccharides and related compounds,

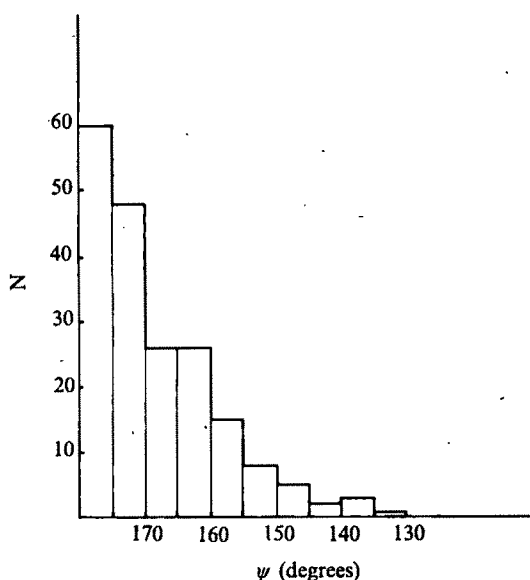


Fig. 3 The histogram from Fig. 2 after correction for the geometric $\sin \psi$ factor.

as listed in Table 1. Whereas the histogram of ψ (Fig. 2) has a maximum at 161°, the application of the $\sin \psi$ correction procedure produces a histogram (Fig. 3) that has a maximum at 180°.

Although distributions of experimental values of hydrogen bond angles at first sight suggest the opposite, we conclude that the experimental distributions, properly treated, are not inconsistent with an assumed preference for linear hydrogen bonds.

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Effect of sliding on surface fatigue

IN hardened steels, sliding friction increases the tendency of loaded line contacts to suffer surface fatigue¹, which can lead to pitting. This note describes an apparatus to study this effect in point contact and gives the first results.

The most convenient test pieces for fatigue studies are bearing balls. Our device consists of a drive ball, *a*, loaded against three radially disposed idler or intermediate balls, *i*, each of which is held in a chuck running on an angular contact ball thrust bearing. A fifth ball, *d*, is driven by these three. The contact angle between all the balls is 45° (Fig. 1).

The five-ball assembly is doubled with the driven balls mounted on opposite ends of a floating shaft and thus located without bearings. On this shaft an aluminium disk, together with two d.c. coils, acts as an eddy current brake. A double-ended motor drives both the input balls. By altering the current in the brake coils various amounts of traction or slip can be achieved at the contacts. Digital tachometers on each shaft, and strain gauges connected to suitable control circuits, automatically maintain the desired value of slip or traction. The load is axially applied by deadweight.

The contact zone between any two balls is a flat circular area. Analysis shows that for this system there is no relative motion in the contact plane between the two surfaces. This device therefore undergoes pure rolling. Sliding only occurs when traction is deliberately applied by the eddy current brake. When this was not energised, no slip could be detected

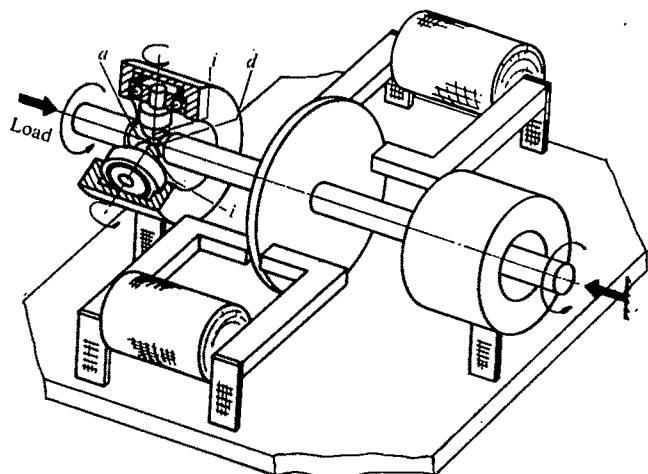


FIG. 1 Schematic twin-head five-ball assembly.

within the limits of accuracy of the measuring instruments (± 1 part in 10^4).

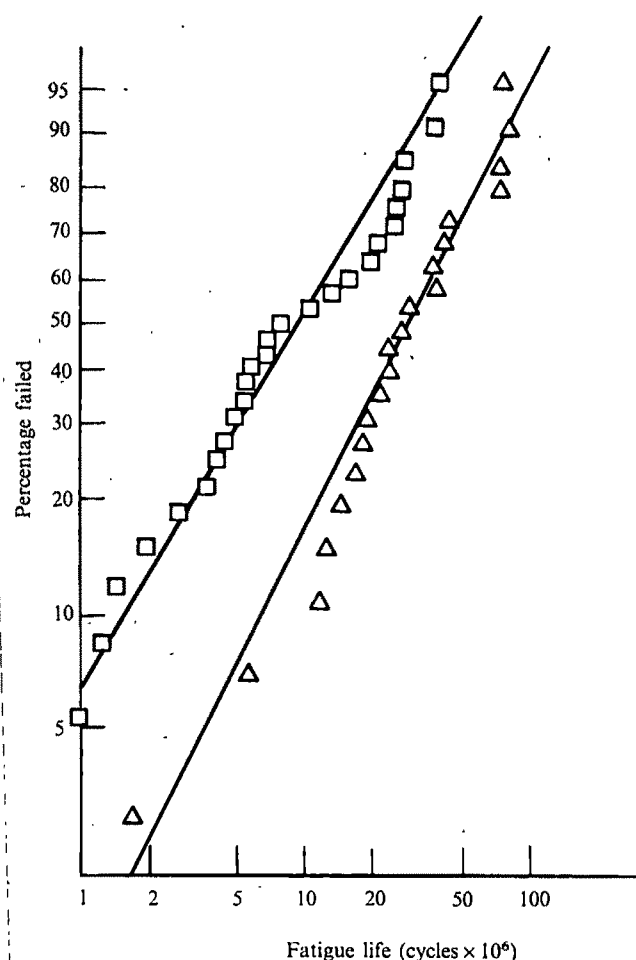


FIG. 2 Weibull plot of results. Δ, Pure rolling; □, 0.35% slide-roll ratio.

Using balls of EN31 (diameter $\frac{5}{8}$ inch) and a synthetic diester lubricant of 7.5 cstokes at 96.8° C (210° F), a standard Weibull plot² was obtained at a maximum Hertz stress of 6 GN m⁻² (Fig. 2).

Tests were then carried out at a slide-roll ratio of 0.35% and a further plot was made. Figure 2 shows that the life decreased by 3.2 times because of this very small amount of sliding.

As no comparable apparatus exists it is not possible to see exactly how these results compare with other published data.

Tests at different contact angles³, which effectively generate different amounts of spin at the contact, have shown that on addition of small amounts of spin, the life is drastically reduced. An increase in mean spin-roll ratio from 0.168% to 0.55% reduces the life by 2.1 times. We note that the lubricant used in this case was a highly refined mineral oil quite different from the synthetic diester which we used.

The effects described here should be studied in more detail now that controlled amounts of sliding can be applied.

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A full report will be published elsewhere.

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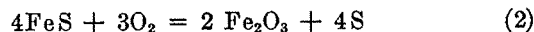
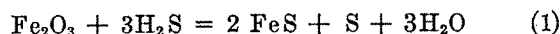
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Is pyrophoric iron sulphide a possible source of ignition?

THERE are many practical environments in which iron oxide, in the form of surface rust, may be exposed to atmospheres containing hydrogen sulphide at various concentrations. Gas lines, chemical plant, distillation units, crude-oil cargo and storage tanks are examples.

We wish to draw attention to the hazard that can arise in such situations because of the formation of pyrophoric iron sulphide, and the possibility that it may act as a source of ignition for inflammable vapours.

The reactions involved in the formation of iron sulphide and its subsequent oxidation on exposure to atmosphere may be represented in a simplified form as follows



Both reactions are exothermic, the ΔH° values for reactions (1) and (2), based on 2 FeS, being about -40 and -151 kcalorie (-168 and -635 kJ) respectively. If reaction (2) can proceed rapidly and with little dissipation of heat, high temperatures can be expected in the material.

We have found that the reactivity of iron sulphide depends on the type of iron oxide from which it is derived. For example, a common form of rust (α -FeOOH) yields on exposure to hydrogen sulphide a material which is particularly reactive. When exposed to air at ambient temperature, the material oxidizes so rapidly that surface glowing and sparking can be seen within seconds. If inflammable vapours come into contact with the material in this condition, the material can act as a source of ignition. We suspect that several recent minor explosions in the cargo tanks of crude-oil tankers may have been caused in this manner. This view is reinforced by the fact that we have been able to demonstrate that pyrophoric iron sulphide can form at partial pressures of hydrogen sulphide corresponding to the values encountered in the ullage regions of crude-oil cargo tanks.

It is fortuitous that in practical situations not all sulphided iron oxide is as reactive as the material described. The reactivity can be influenced by a number of factors. For instance, the progressive buildup of sulphur generated during each sulphidation/oxidation cycle can exert a 'screening' effect, resulting in reduced reactivity of the sulphide so formed. Particle geometry and the presence of occluded materials can also affect reactivity.

Although pyrophoric iron sulphide has, of course, been known for many years¹, the conditions under which it may form and the various factors that can influence its reactivity do not seem to have been fully explored. For this reason its potential danger may often be overlooked.

A fuller account will be given elsewhere.

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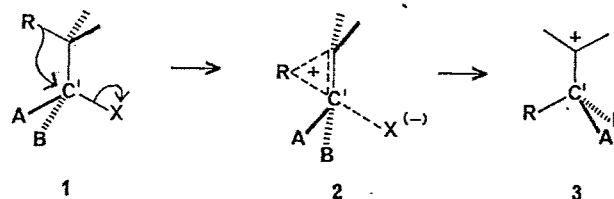
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Stereochemistry of a carbonium ion rearrangement

CONCERTED rearrangements of the carbonium ion kind ($1 \rightarrow 3$) are usually supposed to go with stereochemical inversion at the migration terminus (C^1) and retention in the migrating group (R) (ref. 1). Many chemists have recorded one or other of these results but no one has shown both in one and the same acyclic molecule, largely because



it is very difficult to find a reaction in which both centres are chiral and both survive as chiral in the product. Rigid cyclic systems do show both results at once but this is not very informative as no other outcome is conceivable in a molecule which has no opportunity for internal rotation.

We have been studying² phosphinyl rearrangements (1 , $R = \text{Ph}_2\text{PO}$), attracted by the very high yields, usually more than 95%, of single products formed in these reactions. We wanted to look at the stereochemistry at the migrating phosphorus atom, since it does not follow automatically from the carbon case that retention will be the general rule and also at carbon (C^1 in 1) since we should like evidence on the detailed mechanism of the reaction: whether, for example, the two steps shown in (1) are concerted. This kind of question is worth asking because most accounts of carbonium ion rearrangements assume that electronegative groups like Ph_2PO will not migrate easily since they cannot support the positive charge in the transition state (2).

In one of our reactions² ($4 \rightarrow 5$, $R^1 = R^2 = \text{Ph}$), a chiral migration terminus remained chiral in the product.

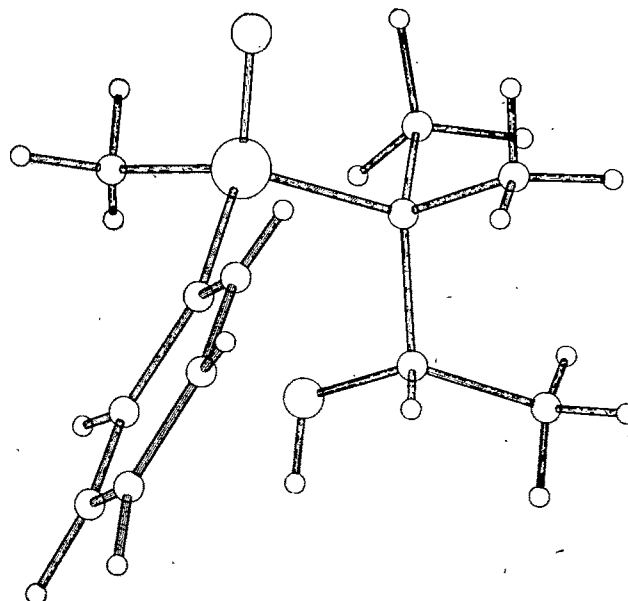


FIG. 1 Perspective drawing of the molecular structure of alcohol ($7a$).

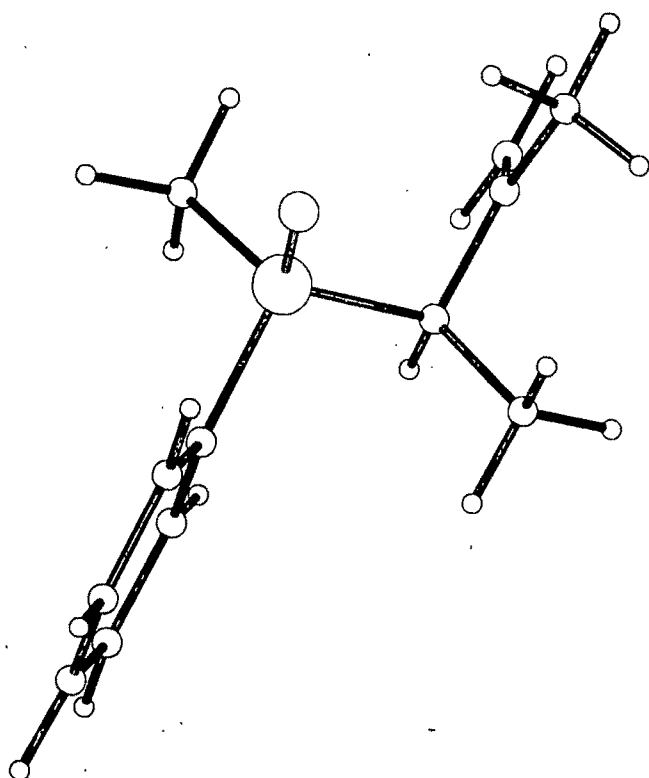
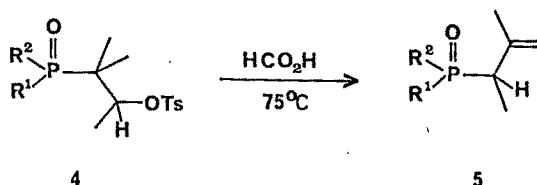
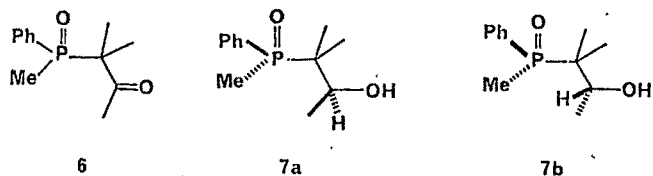


FIG. 2 Perspective drawing of the molecular structure of olefin (9a).

Simply by carrying out this reaction sequence on an analogous molecule with different substituents ($R^1 = \text{Ph}$, $R^2 = \text{Me}$) on phosphorus we have been able to study the stereochemistry at both chiral centres simultaneously and to do so by unambiguous X-ray crystal structure determinations since we need to distinguish diastereoisomers and not mirror images.



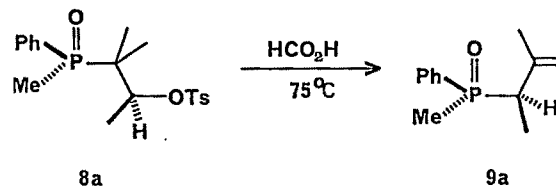
We first synthesised both diastereoisomers of the alcohol (7) by reduction of the ketone (6). One alcohol was easy to make as the bulky reducing agent $\text{LiAlH}(\text{O}i\text{Bu})_2$ gave it in 90% yield. It formed chunky prisms from di-isopropyl ether-chloroform and its structure proved to be (7a) from the X-ray determination (Fig. 1). The other diastereoisomer (7b) was more difficult to make and in the end we separated a 60:40 mixture (from reduction with sodium borohydride



of the ketone (6)) by preparative t.l.c. of the tosylates. Tosylates (8a), made directly from alcohol (7a), and (8b) from the t.l.c. separation, had clearly distinct nmr spectra.

Tosylate (8a) solvolysed in formic acid at 75° C to give an olefin as the major product. This olefin (9a) was very soluble in water and in most organic solvents but formed

long thin trapezoidal prisms from dry 100–120° C petrol ether. Figure 2 shows the result of the X-ray determination of crystal structure. Both structure analyses were based on diffractometer data taken with monochromatised Cu radiation. The phase problem was solved by direct methods and at the current stage of refinement the reliability factors are $R = 0.04$ for the alcohol (7a) and $R = 0.08$ for the olefin (9a).



Some of the other diastereoisomer (9b) of the olefin is also formed in the reaction; the amount varies with time and is about 15% after ten half-lives of the tosylate. If pure olefin (9a) is dissolved in formic acid at 75° C, epimerisation to form (9b) occurs at the same rate as the formation of (9b) in the reaction mixture. So (9b) is not a product of the reaction—it is formed by epimerisation of the true product (9a). Exactly parallel results—formation of (9b) as the only product of the reaction—are found when the minor tosylate (8b) is solvolysed under the same conditions. The tosylates (8) do not epimerise under the reaction conditions.

The reaction is therefore completely stereospecific: configuration is retained in the migrating phosphorus atom and inverted at the migration terminus. This result is consistent with a concerted rearrangement: a less likely but equally valid explanation would be that tosylate leaves to give a cation and rearrangement follows more quickly than rotation of the carbon-carbon bond. Certainly the reaction must be a genuine rearrangement; there can be no question of the phosphinyl group leaving the molecule to rejoin it later at the migration terminus.

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BIOLOGICAL SCIENCES

Repression of RNA transcription during the development of analgesic tolerance to morphine

SEVERAL reports suggest that RNA and protein synthesis are important for the development of morphine-induced analgesic tolerance and dependency^{1–4}. Following up these reports, we have examined the effect of chronic morphine drugging on the intracellular site of RNA synthesis—nuclear chromatin. We found that brain chromatin from rats tolerant to morphine, synthesised RNA more slowly than that from non-tolerant rats⁵. This effect was not caused by the direct

TABLE 1 Chromatin template activity during morphine-induced analgesia

Treatment	pmol of ATP incorporated*
Control	1,136 \pm 47
Morphine (10 mg kg ⁻¹)	1,088 \pm 46

* Results are reported as the mean \pm s.e. for four rats. ATP incorporation by enzyme alone (340 pmol) has been subtracted.

action of morphine and was found to be associated in the chromatin complex with a protein which is believed to regulate RNA synthesis. Since we may have identified, in part, a mechanism by which morphine-induced analgesic tolerance develops, we looked for a chronological relationship between the development of morphine-induced analgesic tolerance and the effect on brain chromatin RNA synthesis.

Male Charles River rats (80–100 g) were divided into two groups: (a) morphine-treated and (b) saline control. Half the daily dose of morphine sulphate was administered subcutaneously twice daily (0800 h and 2000 h) in 0.9% NaCl. Control animals were injected at the same time with 0.9% NaCl. The initial dose of morphine sulphate was 10 mg kg⁻¹ d⁻¹. This dose was increased progressively to 100 mg kg⁻¹ d⁻¹ on the thirteenth day so that the final doses on the first, third, sixth and thirteenth days were 10, 15, 30 and 100 mg kg⁻¹ d⁻¹, respectively. All RNA synthesis and analgesic experiments were begun at 0800 h, 12 h after the last dose of morphine.

Analgesia induced by morphine sulphate was measured by the reaction of animals to being placed on a hot plate using a modification of the method described by Johannesson and Woods⁶. Rats received neither training nor predrug trials before being treated. Twelve hours after their last morphine dose, a subcutaneous 10 mg kg⁻¹ dose of morphine sulphate was given to test and control animals. After 45 min the animals were tested for analgesia. Endpoint responses were paw licking or jumping out of the cylinder with a cut-off time of 30 s. The observer was kept unaware of the treat-

ment for each group. Analgesia was registered as a delay in response to the hot plate while animals tolerant to morphine responded very rapidly.

Nuclei were isolated from individual rat brains using the methods we described previously for pooled rat brains⁶. Chromatin was extracted from rat brain nuclei according to the procedure of Paul and Gilmour⁷, and assayed for its capacity to promote RNA synthesis in the presence of *E. coli* DNA-dependent RNA polymerase according to the methods of Chamberlin and Berg⁸. The reaction mixture (0.5 ml) contained 10 μ M of Tris-HCl (pH 7.9), 1.0 μ M MgCl₂, 3.0 μ M β -mercaptoethanol, 134 μ g of RNA polymerase, 10 μ g of chromatin from either control or morphine-treated animals and 0.1 μ M of UTP, CTP, GTP and ATP (0.1 μ Ci ¹⁴C-8-ATP, New England Nuclear Corp.). Incubation was for 10 min at 37° C. The reaction was stopped by addition of an equal volume of cold 10% trichloroacetic acid (TCA). The precipitate was collected on Millipore filters, air dried, and counted in a toluene-PPO-POPOP cocktail using scintillation spectrometry.

To determine whether the decrease in rat brain chromatin template activity might be related to morphine-induced analgesia, brain chromatin was analysed from rats which had received 10 mg kg⁻¹ of morphine sulphate subcutaneously. At 60 min (a time when all the rats were analgesic) brain chromatin was extracted and its capacity to incorporate ¹⁴C-ATP into RNA measured. Table 1 shows that brain chromatin from both control and morphine-treated rats incorporated equivalent amounts of ¹⁴C-ATP into RNA even though the morphine-treated animals were analgesic.

The development of analgesic tolerance to the morphine regimen used in these experiments is shown in Fig. 1. Rats tested 1 d after morphine treatment seemed to exhibit as much analgesia to 10 mg kg⁻¹ of morphine sulphate as saline-treated rats. Analgesic tolerance became significant at 3 d and was maximal by the sixth day.

Figure 1 also shows the development of the brain chromatin depression during the same morphine regimen. After 1 d, no significant change in chromatin template activity was noted. By the third day the template activity in the morphine-treated rats was significantly depressed and this depression was maximal by the sixth day.

To examine further the relationship of analgesic tolerance and chromatin template activity, we performed hot plate and chromatin template assays at various times after the morphine regimen was stopped. Figure 2 shows the duration of analgesic tolerance produced by the 13-d morphine sulphate regimen. Seven days after the morphine regimen was discontinued analgesic tolerance was still maximal. On the twenty-eighth day, tolerance appeared to be less, but was still significantly different from control. By the thirty-fifth day, no significant analgesic tolerance was detected.

Figure 2 also shows the duration of the chromatin depression produced by the 13-d morphine regimen. Seven days after the morphine treatment was stopped, chromatin template activity was still maximally depressed. However, by the twenty-eighth day, the template activity of the morphine-treated group had increased to the level of the controls.

Our results do not support any relationship between analgesia and brain chromatin template activity, since the chromatin depression was absent during analgesia and yet maximal during analgesic tolerance.

The onset of the depressed chromatin template activity and the onset of analgesic tolerance during chronic morphine treatment seem to be related. Both became significant by the third treatment day and were maximal by the sixth day. On the other hand, when morphine treatment was stopped the depression of template activity had disappeared by the twenty-eighth day, while analgesic tolerance was still significant and did not disappear until the thirty-fifth day after

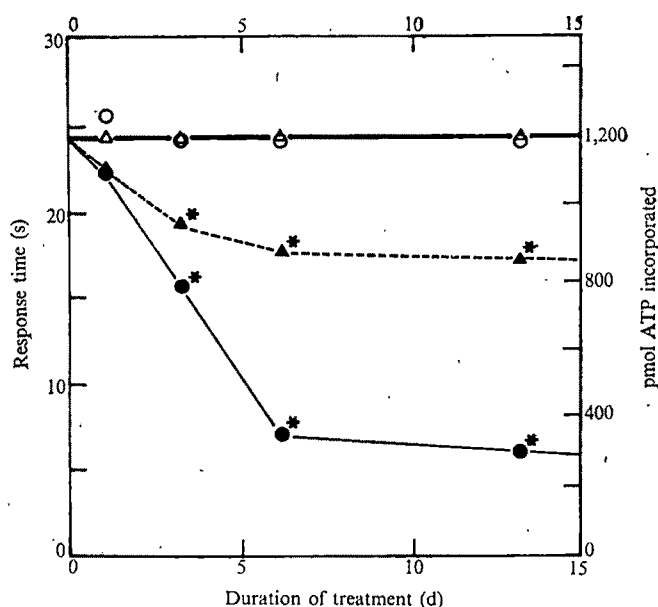


FIG. 1 Development of morphine-induced analgesic tolerance and depression of brain chromatin template activity. Each circle (○, control; ●, morphine) is the mean response time of thirteen to twenty-five rats. Each triangle (Δ, control; ▲, morphine) is the mean template activity of three to ten rat brains. The greatest number of rats were used at days 1, 3 and 6. * Denotes values which are significantly different ($P < 0.05$) from their respective control by Student's *t* test⁹.

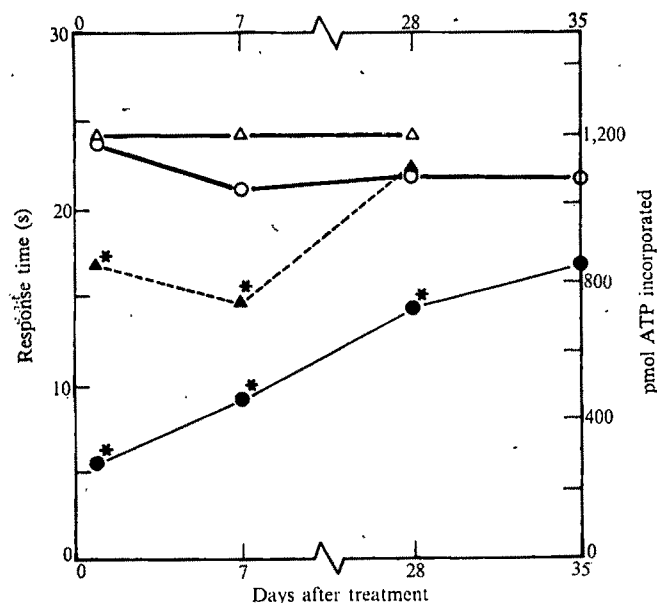


FIG. 2 Disappearance of morphine-induced analgesic tolerance and depression of brain chromatin template activity. Each circle (○, control; ●, morphine) represents the mean response time of ten to twenty-five rats. Each triangle (△, control; ▲, morphine) represents the mean template activity of three to eight rat brains. * Denotes values which are significantly different ($P < 0.05$) from their respective control by the Student's t test⁹.

treatment. Although chromatin template depression and analgesic tolerance did not disappear together, this does not negate a possible relationship between them. If the change in nuclear chromatin template initiates the mechanism(s) producing analgesic tolerance, then one would expect such tolerance to disappear with or after the chromatin effect.

In conclusion, we found that changes in brain chromatin template activity correlated chronologically with the onset and disappearance of morphine-induced analgesic tolerance. However, it is quite possible that the depression of chromatin template activity may be related to morphine dependency, or perhaps, it is related to both phenomena. The latter possibility seems quite possible, in light of the fundamental role of chromatin in cellular RNA synthesis and the difficulty of separating morphine-induced analgesic tolerance and dependency.

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Defective rRNA synthesis in human leukaemic blast cells?

RECENT studies suggest that processing of ribosomal precursor RNA might be impaired in blast cells of acute leukaemia (AML)^{1,2}. Following incubation with ³H-methyl-methionine blast cells of human acute leukaemias partially failed to methylate the 45S and 32S ribosomal precursors¹. Using autoradiographic techniques, previous studies have also shown an increase in intranuclear RNA and decreased cytoplasmic RNA in these cells³. More recently, competitive hybridisation was performed with normal lymphocyte DNA and 35S-55S RNA fractions extracted from both acute leukaemia blast cells and normal PHA-stimulated lymphocytes. Using incubations with actinomycin D these experiments have led to the conclusion that leukaemic blast cells synthesise heterogeneous nuclear RNA molecules which are not transcribed in PHA-stimulated lymphocytes and it was also suggested—in contrast to the earlier data—that the amount of unmethylated unprocessed rRNA precursor was small in leukaemic blast cells⁴.

Studies in this laboratory have yielded highly specific ³²P-labelling of nuclear and ribosomal high molecular weight RNA using a HEPES-buffered, phosphate-free medium⁵.

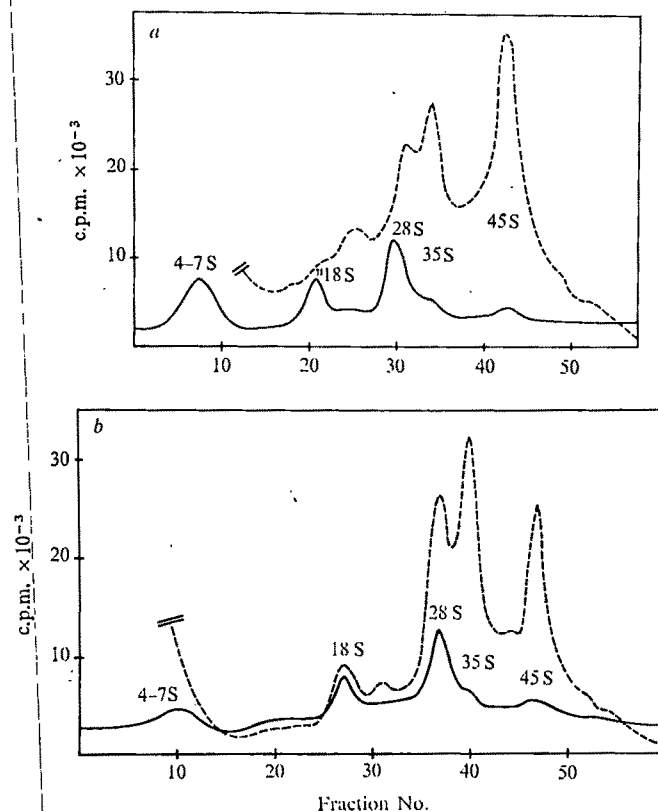


FIG. 1 a, Sucrose density gradient centrifugation of nuclear RNA from acute myeloblastic leukaemia cells. The cells were labelled for 6 h in a phosphate-free medium⁵. Nuclei were isolated by homogenisation in 5% citric acid and purified by sucrose step gradients⁶. The RNA was extracted by the hot phenol-SDS-method⁷ at 65° C for 5 min. RNA (1 mg) was loaded on a 6%-45% (w/w) sucrose density gradient (Beckman Density Gradient Former) and centrifuged for 16 h at 26,000 r.p.m. (3° C, SW27 rotor). The RNA was fractionated with an ISCO Model 640 Density Gradient Fractionator and aliquots of 20 μ l were counted in Bray's solution in a Packard Model 3380 Tricarb Liquid Scintillation Spectrometer to determine the distribution of radioactivity. b, Sucrose density gradient centrifugation of nuclear RNA from PHA-stimulated normal lymphocytes. Lymphocytes were derived from normal donors by separation on nylon fibre columns¹⁰. PHA stimulation was done according to the method of Cooper¹⁰. For details of RNA extraction and separation see (a).

TABLE 1 Comparative specific activities of ^{32}P -labelled nuclear 45S RNA and 28S rRNA from PHA-stimulated lymphocytes and human leukaemic blast cells

Tissue	c.p.m. per mg RNA		Specific activity
	Nuclear 45S RNA	28S rRNA	45S nRNA 28S rRNA
AML (Patient N., H.)	3.2×10^8	4.7×10^8	68.1
AML (Patient N., K.)	2.8×10^8	2.4×10^8	116.2
AML (Patient L., J.)	1.5×10^9	9.3×10^8	161.0
PHA-stimulated normal lymphocytes			
Experiment 1	1.5×10^9	1.6×10^8	9.4
2	1.2×10^9	1.7×10^8	7.1
3	1.3×10^9	1.4×10^8	9.3

The conditions were standardised in that each gram of packed leukaemic cells derived from heparinised blood of untreated leukaemia patients was incubated at 37° C with 50 mCi ^{32}P -orthophosphate using a 1:100 ratio (w/w) of cells and medium. Nuclei were isolated after 6 h using 5% citric acid to prevent RNase degradation as described by Dounce⁶. The purified nuclear pellet was subjected to controlled hydrolysis with 1.0 mg highly purified DNase (Boehringer) per g nuclei in 1.5 ml 0.5 M NaCl, 0.05 M MgCl_2 , 0.001 M Tris, pH 7.4, at 22° C for 3 min. Buffer (40 ml) containing 0.1 M NaCl, 0.01 M Na acetate, 0.001 M MgCl_2 , pH 5.1, was added to 1 g of packed nuclei and the suspension was made 0.34% in sodium dodecylsulphate. The mixture was shaken with hot phenol at 65° C for 5 min as described by Scherrer and Darnell⁷. After two re-extractions with hot phenol, undegraded, highly labelled nuclear RNA was precipitated with two volumes of ethanol as demonstrated by sucrose density gradient centrifugations (Fig. 1a and b). A marked label was found in the 45S region and, in all experiments, only the three peak fractions were collected.

In a different batch, 1 g of cells derived from the same patient was incubated for 9 h using identical conditions and cytoplasmic RNA was extracted from a ribosomal preparation as described previously⁸. To eliminate the possibility of degradation, rRNA preparations were only used when they met the basic requirements of purity and quality as outlined by Attardi and Amaldi⁹.

Lymphocytes stimulated with PHA were prepared as described by Cooper¹⁰. In all experiments with both leukaemia cells and PHA-stimulated lymphocytes, nuclear 45S RNA and 28S rRNA were characterised by the determination of specific activities, nucleotide composition analyses, and oligonucleotide frequency studies after complete digestion of the RNA fractions with pancreatic RNase. The techniques of column chromatography on DEAE-Sephadex A25 and high voltage paper electrophoresis have been described elsewhere^{11,12}. The details of comparative structural studies of high molecular weight nuclear and rRNA species from various human leukaemias and lymphomas will be reported elsewhere (S.S., K.P.B., J.K., C.G.S. and H. Busch, unpublished).

Figure 1 shows the distribution of radioactivity in nuclear RNA fractions when separated on 6%–45% sucrose density gradients (pH 5.1) and fractionated into 58–60 portions of 0.6 ml. Some differences were found between the proportion of label in 45S, 35S and 28S RNA with a higher percentage of label in 45S in the case of AML (Fig. 1a and b). High specific activities up to 1.5×10^9 c.p.m. per mg RNA were

determined for the 45S fraction both from PHA-stimulated normal lymphocytes and leukaemic blast cells (Table 1). No major differences were found in nucleotide composition analyses (all run in triplicate) between two of three cell lines of human leukaemia and the transformed normal lymphocytes (Table 2). Table 2 also indicates that the RNA found in the 45S fraction was mainly of the preribosomal type with a G+C/A+U ratio of 1.96–2.00. Table 1 summarises the specific activities of nuclear 45S RNA and ribosomal 28S RNA from three individual experiments each with acute myeloblastic leukaemia cells and PHA-stimulated normal lymphocytes.

The striking differences found in the ratios of the specific activities, as well as the demonstration of very small amounts of label in 28S rRNA of blast cells clearly indicate a failure of rRNA synthesis in the leukaemia cells. Although there were some consistent differences in the ^{32}P -nucleotide compositions with equal labelling of guanylic and cytidylic acid in the case of the blast cells (the values for a number of tissues reported by Attardi and Amaldi⁹ correspond to those found for PHA-stimulated lymphocytes in our studies) no significant structural differences were found between 28S rRNA of leukaemic and non-leukaemic cells when the frequencies were determined for the partial sequences A-Cp, A-Up, G-Cp, G-Up, A-A-Cp, (A, G)-Cp, A-A-Up, G-G-Cp, G-A-Up, A-G-Up and G-G-Up (S.S., K.P.B., J.K., C.G.S., and H. Busch, unpublished).

These results, based on high specific labelling of nuclear and cytoplasmic RNA species, support the previous findings of a block in processing of the ribosomal precursor to the RNA of the mature ribosome in blast cells of human acute leukaemia. This condition, however, may not be unique to leukaemic blast cells since it has also been pointed out that processing of the ribosomal precursor may be different in actively growing cells, resting cells and cells under transition from the resting state to active growth¹³. It may be of interest that with the use of the same techniques we found similar small amounts of label in cytoplasmic RNA in acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL). Conversely, high specific activities of 28S rRNA were determined in two cases of leukaemic lymphosarcoma and in a Burkitt lymphoma cell line (unpublished).

The transformation of chronic lymphocytic leukaemia into lymphosarcoma which has been reported many times may be, on a cellular level, characterised by an increased processing of preribosomal RNA. With our preparations of RNA from a monosome-polysome pellet we never found disproportionate labelling of 18S and 28S RNA with rela-

TABLE 2 ^{32}P -Nucleotide compositions of nuclear 45S RNA and 28S rRNA from PHA-lymphoblasts and leukaemic blast cells

Tissue	28S rRNA					45S nRNA				
	A	U	G	C	s.e.	A	U	G	C	s.e.
AML (Patient N., H.)	15.7	16.2	34.6	33.6	<0.6	14.5	19.1	32.8	33.6	<0.5
AML (Patient N., K.)	15.7	17.4	33.2	33.7	<0.4	13.8	18.1	34.2	33.9	<0.4
AML (Patient L., J.)	17.1	15.4	33.9	33.6	<0.3	18.1	21.5	29.8	30.7	<0.6
PHA-stimulated lymphocytes	16.4	15.9	35.8	31.9	<0.3	14.5	20.1	32.5	32.8	<0.5

tively small amounts of label present in the 18S rRNA. It has been postulated that the assembly of ribosomes in normal and also leukaemic lymphocytes would be controlled by such a 'wastage' of 18S rRNA^{14,15}. These studies were, however, based on RNA extractions from unfractionated lymphocytes and showed very low label in the 18S fraction of resting normal and leukaemic lymphocytes.

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Presence of a large poly(rC) tract within the RNA of encephalomyocarditis virus

THE replication of the RNA-containing bacteriophages has been found to involve specific replicases, partly coded by the phage genome (reviewed in refs 1 and 2). The replication proceeds by way of a partially double-stranded intermediate form, and the replicases must presumably recognise the 3'-terminal regions of the RNA (+) and (-) strands as start points for copying^{1,2}. There is also evidence that the Q β replicase recognises a specific internal region of the Q β RNA molecule, which is involved in forming a replicative complex³. Replication of the smallest animal RNA viruses, the picornaviruses, also requires specific replicases, and has a mechanism⁴⁻⁷ which is not dissimilar to that of the RNA phages, as far as it has been elucidated. We have been studying nucleotide sequences of regions of a picornavirus RNA which might be specifically recognised by its replicase, or have specific roles in the replicative process, in the same way that such sequences have been characterised in the RNA phages¹⁻³.

Encephalomyocarditis (EMC) virus, which is typical of the cardiovirus group of picornaviruses, contains a continuous single-stranded RNA molecule of molecular weight around 2.7×10^6 , about 7600 nucleotides in length (refs 8 and 9 and D. Frisby, unpublished). We have found a region of 95-100 nucleotides in the interior of the molecule, con-

taining 85-90 C residues. Here we describe the isolation and characterisation of this poly(rC) region, and discuss its possible significance.

EMC virus was cultivated in Krebs ascites cells in the presence of ³²P-orthophosphate, and radioactive virus was purified from the cell lysate in the way described in the legend to Fig. 1. The RNA was extracted from the purified virus by phenol-chloroform treatment. Subsequent sucrose gradient centrifugation of the RNA revealed a single peak, shown in Fig. 1, with a sedimentation coefficient of about 37S, corresponding to the size previously reported for EMC RNA (refs 8 and 9 and D. Frisby, unpublished). Electrophoresis of this RNA peak through 4% polyacrylamide gels containing formamide yielded a single high molecular weight band, demonstrating the absence of any hidden breaks in the purified material. We therefore suggest that the products

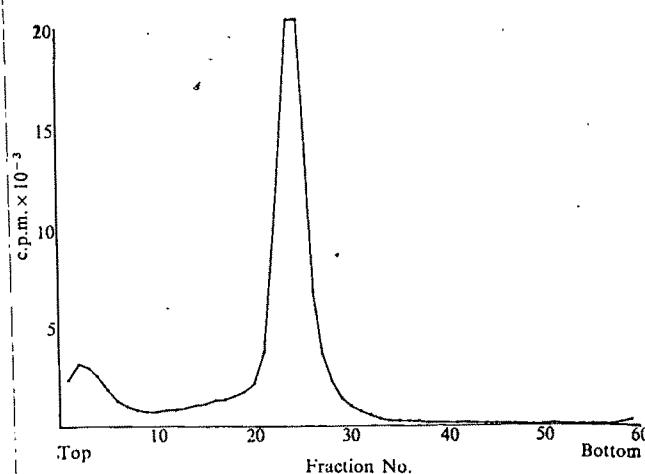


FIG. 1 Sucrose gradient profile of ³²P-labelled EMC RNA. The RNA was prepared as follows. A 100 ml suspension of washed Krebs ascites cells in HB-NE medium (New Earle's medium, containing in g l⁻¹: NaCl, 6.28; KCl 0.37; MgSO₄ · 7 H₂O, 0.18; phenol red, 0.0009; glucose, 1.8; CaCl₂ · 6 H₂O, 0.18; NaHCO₃, 3.3; penicillin G, 0.06; and streptomycin, 0.06; buffered to pH 7.5 with HEPES) containing 1.5×10^7 cells ml⁻¹ was infected with EMC virus at a multiplicity of 10 PFU per cell. To enable viral multiplication, the infected cell suspension was allowed to stand at 22° C for 0.5 h, then gently shaken in a 1 l flask for 3 h at 37° C. ³²P-orthophosphate was then added to a concentration of 0.1 mCi ml⁻¹, and shaken for a further 16-18 h during viral multiplication. The resulting lysate was centrifuged at 16,000g for 0.5 h at 4° C (MSE centrifuge) to remove cell debris. Labelled virus was pelleted from the supernatant by centrifugation (110,000g) for 2 h at 4° C (in the Beckman L2-50 ultracentrifuge) and the pellet partially resuspended in 1 to 2 ml of PBS-A buffer (containing in g l⁻¹: NaCl, 8.0; KCl, 0.2; Na₂HPO₄ · 1.15 g; KH₂PO₄, 0.2, pH 7.3), then left overnight at 4° C. Several volumes of PP8 buffer (0.1 M NaCl, 0.1 M K₂HPO₄ and 0.2 M Na₂P₂O₇ · 10 H₂O, pH 8.0) were added to this suspension to solubilise the virus, and the total volume was made up to 25 ml with additional PBS-A. The suspension was then layered onto a 10 ml sucrose cushion (29% sucrose in PBS-A) and centrifuged at 110,000g as above, but for 3 h. The second virus pellet was resuspended in PBS-A together with an equal volume of PP8, as described above, and layered onto an 11 ml 10.2-22.4 % isokinetic sucrose gradient in PBS-A buffer, and centrifuged for 75 min at 36,000 r.p.m. at 4° C in the SW41 rotor. Over 95% of the radioactivity on the gradient was always found in the 160S virus peak, which was extracted with equal volumes of Tris buffer-saturated phenol (pH 7.5) and chloroform, in the presence of 0.5% sodium dodecyl sulphate. The ³²P-labelled EMC RNA was precipitated from the aqueous phase by the addition of 0.5 mg of unlabelled carrier RNA, 0.1 volume of 2 M sodium acetate and 3 volumes of ethanol. This RNA was sedimented through a further similar sucrose gradient for 3.5 h, the profile of which is shown above. About 50 μg of labelled RNA with a specific activity of $0.5-0.8 \times 10^6$ c.p.m. μg⁻¹ was usually obtained.

TABLE 1 Analysis of the large T_1 RNase resistant oligonucleotides from EMC RNA. The products arising upon complete pancreatic RNase digestion of fragments A_0 - A_3 and α (Fig. 3) were excised, and the radioactivities of the spots on paper were determined directly by scintillation counting, enabling the quantities of the products, relative to Gp, to be estimated (column 3). All the estimates are calculated from the mean values of three determinations, and enable the lengths of the oligonucleotides to be approximately estimated, given in column 4. These lengths are consistent with the mobilities of the fragments relative to each other, and to the electrophoretic front in 10% polyacrylamide, and suggest that the fragments are homogeneous. This is also supported by the amounts of the fragments released upon hydrolysis of the RNA, about one mole of each per mole of RNA (Column 5). Their yields were determined by counting a small aliquot of an RNA digest (0.8%) before gel electrophoresis, and then counting the fractionated fragments following their elution from polyacrylamide onto DEAE-paper discs (this part of the elution procedure is quantitative since in multiple experiments the amount of residual ^{32}P counts remaining in the gel slice compared with that eluted onto the DEAE-paper discs was always less than 0.5%. No leakage of ^{32}P into the anode buffer compartment was detectable). Taking the length of the RNA as 7600 nucleotides (refs 8 and 9 and D. Frisby, unpublished), the total radioactivity of the RNA digest applied to the gel may be calculated in terms of c.p.m. per nucleotide, and thus also the expected radioactivities of fragments of these sizes, if each is present once. The observed molarities may then be calculated from comparison of the observed radioactivities with the expected radioactivities (Columns 5 and 6). In a typical experiment, 8.6×10^6 c.p.m. of digested EMC RNA were applied to a gel slab. The radioactivities of the fragments eluted were determined to be 92,000 c.p.m. (A_0), 36,700 c.p.m. (A_1), 46,300 c.p.m. (A_2) and 39,400 c.p.m. (A_3).

Fragment	Analysis of fragment			Chain length and yield of fragment		
	Oligonucleotide	found	Yield suggested	Suggested length (Nucleotides)	Abundance in EMC RNA (moles per mols RNA) Found	Suggested
A_0	AAC	0.7	1	95-100	0.9	1
	AC	0.9	1			
	G	1.0	1			
	C	87	85-90			
	U	4.1	4			
A_1	AAAAC	0.8	1	36-41	1.0	1
	AAU	1.5	2			
	AAC	1.5	2			
	AU	2.6	3			
	AC	1.2	1			
	G	1.0	1			
	C	5.9	6-8			
	U	4.5	4-7			
A_2	AAU	1.5	2	26-31	1.4	1
	AAC	1.5	2			
	AC	1.0	1			
	G	1.0	1			
	C	7.9	6-9			
	U	6.2	5-7			
A_3	AAAAC	1.0	1	27-31	1.2	1
	AAU	0.8	1			
	AAC	1.0	1			
	G	1.0	1			
	C	7.1	6-8			
	U	8.5	7-9			
α	?	—	—	}	0.3	
	?	—	—			
	?	—	—			
	AAG(?)	0.1	—			
	AAU	0.2	—			
	AAC	0.3	—			
	AG	0.3	—			
	AU	0.5	—			
	AC	0.7	—			
	G	1.0	—			
	C	12.4	—			
	U	2.0	—			

arising from subsequent nuclease digestion of this RNA cannot be derived from any contaminating low molecular weight material within the virus particles, whether or not associated with the EMC RNA. ^{32}P -labelled EMC RNA was completely digested with T_1 RNase, and the resulting products were fractionated through a large slab of 10% polyacrylamide gel containing 7 M urea, as described in the legend to Fig. 2. The products were located by autoradiography (Fig. 2).

The majority of the oligonucleotides migrate close behind the electrophoretic front, and are in the size range 1-25 nucleotides, as expected. Three larger products, A_1 , A_2 and A_3 are also found, of approximately 25-40 nucleotides. This also corresponds to the expected abundance of oligonucleotides of this size arising upon T_1 RNase digestion of an RNA of the size and composition of EMC. A further product, A_0 , however, containing 95-100 nucleotides by

analysis, is also present. This is much larger than any oligonucleotide which would be expected to arise from an RNA of 7,600 nucleotides in a random sequence, since the probability of a section of this length lacking any G residues is vanishingly small. Finally a further band, α , apparently slightly larger than A_0 , is found in small and variable amounts among the digestion products.

We have analysed the fragments A_0 - A_3 and α by further digestion with pancreatic RNase. The results of these analyses are reported in Table 1, and the fractionation of the products is shown in Fig. 3. The gel fractionation indicates that fragment A_2 must be a few nucleotides larger than A_3 , but our analyses are not sufficiently precise to show the exact size difference. Fragments A_1 , A_2 and A_3 display no unusual structural features, as judged from the results of these partial analyses. In particular, there is no preponderance of any one nucleotide in them.

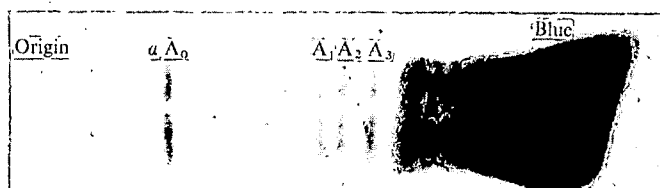


Fig. 2 Fractionation of the products arising upon complete hydrolysis of EMC RNA with T_1 RNase. $2-40 \times 10^6$ c.p.m. of ^{32}P -labelled EMC RNA (5–50 μg , containing 500 μg unlabelled carrier tRNA) was dissolved in 0.02 ml of 0.02 M Tris HCl pH 7.4; 0.002 M EDTA (low salt buffer) containing T_1 RNase (Sankyo Ltd., Japan) at an enzyme: substrate (w/w) ratio of 1:40. Digestion was continued for a further 1 h at 37°C . Alternatively digestion was carried out in 0.02 M Tris HCl-0.02 M MgCl_2 (high salt buffer) at pH 7.5. No difference in the digestion pattern was observed. The RNA digest was mixed with an equal volume of 7 M urea containing 60% sucrose and a trace of bromophenol blue, and layered into a $0.1 \times 1 \times 5$ cm pocket at the top of a 10% polyacrylamide gel slab (measuring $0.3 \times 15 \times 40$ cm) made up in the following buffer: 0.1 M Tris borate, pH 8.3; 0.0025 M EDTA and 7 M urea. Electrophoresis was carried out in the cold for 16–18 h at 500 V and 30 mA. The radioactive bands were located by autoradiography, as shown above, and excised using the autoradiograph as a guide. Fragments A_0 – A_3 and α were eluted electrophoretically onto DEAE-cellulose paper discs which were washed in ethanol and counted to estimate the molar yields of the fragments (see legend to Table 1). Elution of the labelled fragments from the discs was performed using freshly-prepared 30% triethylammonium carbonate, pH 10, as described by Adams *et al.*²⁵

In contrast, the very large product A_0 contains 85–90 C residues, together with 3 As, 4 Us and the expected 3' terminal Gp. The estimated size of 95–100 nucleotides derived from this analysis is consistent with its position on the 7 M urea gel relative to the other fragments A_1 – A_3 . Estimates of the sizes of these are also included in Table 1. We have also determined the yields of the fragments α and A_0 – A_3 in terms of the numbers of moles of each which arise from each mole of EMC RNA upon enzymatic hydrolysis. These values are reported in Table 1. It may be seen that all of the fragments except α are equimolar, and each seems to occur once in the molecule.

The analysis of band α is also listed in Table 1. From its position on gel electrophoresis, the material contained in it is a few nucleotides longer than the fragment A_0 . On the basis of a length of 100 nucleotides it is present in roughly 0.3 M amounts in digests of the RNA. It has a complex composition. Like A_0 , it is very rich in C, but this may arise by contamination with A_0 because of 'tailing' of the latter fragment. It also contains more than one product terminating in Gp, in addition to Gp itself, which suggests that it might be a mixture of two or more components, each present in very small amounts in the digest. These might arise because of incomplete hydrolysis of a small proportion of the RNA, and this view is supported by the variable amounts of α present in digests. Perhaps this is due to incomplete deproteinisation of the RNA affording specific nuclease-resistant areas. This is purely speculative, however.

So far we have no direct evidence about the location of the poly(rC) tract within the EMC RNA; however an indirect argument suggests that it might be situated close to one of the termini. The aggregate molecular weight of the EMC virus-specified proteins has been estimated to be about 2.3×10^5 (ref. 10). The molecular weight of the RNA necessary to code for these proteins would be about 2.3×10^6 . Since the molecular weight of the EMC RNA is around 2.7×10^6 , there are roughly 1,000 nucleotides of untranslated RNA within the EMC viral genome. These

untranslated regions must be situated at the termini of the RNA, since the initial translation product is a single polypeptide¹⁰; that is, there are no untranslated areas between the cistrons. The poly(rC) tract would code for a polyproline region if it were translated. The amount of proline present in the proteins of the EMC viral capsid is relatively small¹¹; however, we cannot exclude the possibility that a polyproline tract is present in some other virally-specified protein within the host cell cytoplasm, although this seems unlikely. This suggests that the poly(rC) region is probably untranslated, and so occurs fairly close to one or other of the termini of the RNA molecule. If so, it would account for roughly 10% of the untranslated nucleotides.

Poly(rC)-enriched regions do not exist in bacterial tRNAs¹² or rRNAs^{13,14}, nor have they been detected within small phages such as Q β or R17 (refs 1 and 2 and A. G. Porter, unpublished). In the case of Q β RNA (A. G. Porter, unpublished), oligonucleotides resulting from complete T_1 RNase digestion were fractionated by electrophoresis followed by homochromatography^{15,16}. None of the largest T_1 RNase fragments (15–25 nucleotides) were found to be unusually rich in any one nucleotide. Preliminary analyses of the longest oligonucleotides arising from T_1 RNase digestion of influenza viral RNA and tobacco mosaic viral RNA^{17,18} have also not revealed poly(rC). Since poly(rC) areas have not been detected in any other naturally occurring RNAs, can any specific function be attributed to this region within the EMC RNA? Two observations suggest that the poly(rC) might be part of a site recognised by the EMC viral replicase at some stage in the replicative process. First, it has recently been reported¹⁹ that a partially purified EMC viral replicase preparation will synthesise poly(rG) from a poly(rC) template. Second, the Q β replicase, which has subunits of very similar size to those of the EMC replicase¹⁹, can also synthesise poly(rG) using a poly(rC) template, but cannot use other homopolymers or natural nucleic acids as templates, except the Q β RNA itself^{20,21}. As mentioned above, however, the Q β RNA does not contain a T_1 RNase-resistant tract of poly(rC) comparable in length with that of EMC RNA (refs 1 and 2 and A. G. Porter, unpublished), and therefore the significance of this observation remains unclear. The suggestion that the poly(rC) region in EMC RNA might function in replication is reinforced if, as seems likely, the poly(rC) tract lies close to one of the termini in a non-coding area of the RNA.

About 60% of the nucleotides in the EMC RNA participate in secondary structure when the RNA is in free solution (D. Frisby and R. Cotter, unpublished). If the poly(rC) tract is involved in this secondary structure, a corresponding area rich in poly(rG) would be expected in the EMC RNA. Complete digestion of the RNA with pancreatic RNase, however, does not give products longer than 15–20 nucleotides (our unpublished results), excluding the presence of long uninterrupted tracts of poly(rG). Therefore any secondary structure involving the poly(rC) region would contain substantial mismatching, although no conclusions can yet be drawn regarding the presence or absence of such secondary structure.

No products containing extended tracts of poly(A) occur among the large oligonucleotides arising from T_1 RNase digestion of EMC RNA in either high salt or low salt conditions. These ought to be detectable in the same way as other large oligonucleotides, if present, and it therefore seems that the RNA, at least within the virus particle, lacks any appreciable poly(A). Similar findings have been reported for the closely related cardiovirus Mengo²², where the maximum length of the poly(A) tracts has been estimated to be about 16 nucleotides. We have not yet systematically examined the smaller EMC RNA oligonucleotides in this size range, and therefore we do not know whether any

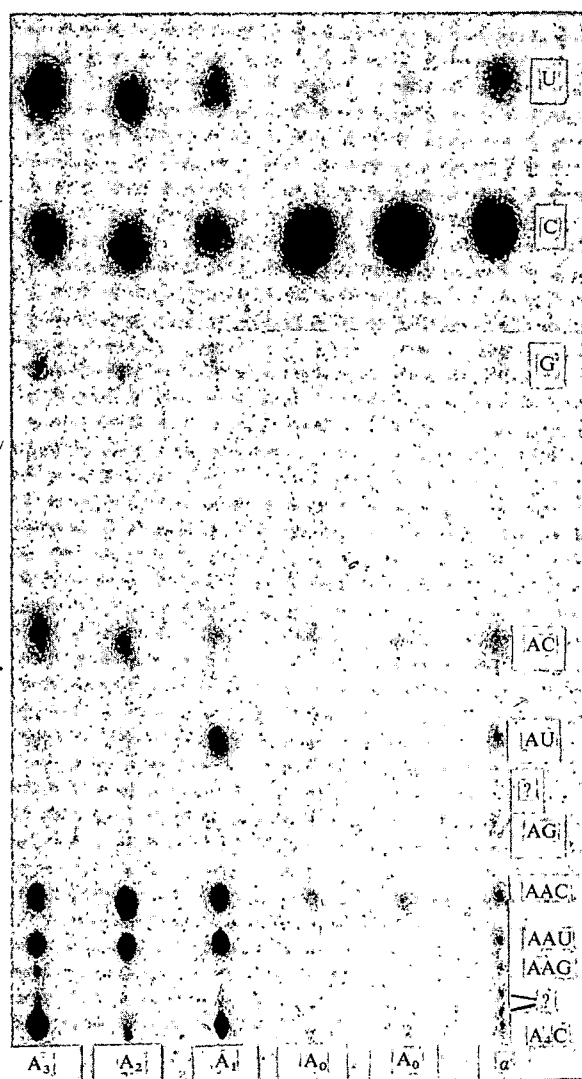


FIG. 3 Fractionation of the products of pancreatic RNase digestion of the large fragments from EMC RNA. The fragments A_0 - A_3 and α (Table 1) were completely digested with pancreatic RNase as described by Brownlee *et al.*^{15,16} and the products fractionated by electrophoresis on DEAE paper at pH 3.5 (0.5% pyridine-5% acetic acid). The positions of the radioactive spots were determined by autoradiography, as shown above, and the oligonucleotides quantified by counting and eluted^{15,16}. In most cases, the pancreatic oligonucleotides could be identified from their positions on the autoradiograph. In all cases, however, firm identification was made (Table 1) by total alkaline hydrolysis of every oligonucleotide, followed by fractionation of the products by electrophoresis on Whatman No. 52 paper at pH 3.5 (refs. 15 and 16). In one experiment, oligonucleotides arising from pancreatic RNase digestion of fragments A_0 - A_3 were separated by two-dimensional electrophoresis (on cellulose acetate at pH 3.5 and DEAE paper in 7% formic acid^{15,16}). The pancreatic RNase digest of fragment A_0 was fractionated in two tracts in order to obtain a mean count of each oligonucleotide present.

short poly(A) stretches are present in the EMC RNA. The cardioviruses therefore seem to differ in this respect from polio, an enterovirus, which has been reported to contain about 50 (ref. 23) or 90 (ref. 24) A residues at its 3'-terminus.

We are now examining the RNAs of a number of other picornaviruses for the presence of poly(rC) tracts, and attempting to determine the location of the poly(rC) region in the EMC RNA directly by sequencing.

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In vivo and in vitro effect of α -amanitin on preimplantation mouse embryo RNA polymerase

THE effects of various metabolic inhibitors, including actinomycin D, puromycin, cycloheximide, mitomycin C, and fluorophenylalanine, on preimplantation mouse embryos have been previously studied¹⁻⁶, to investigate when RNA synthesis begins in preimplantation embryos and what types of RNA are synthesised during the various stages of development. Monesi and Salfi³ originally reported that there was no appreciable overall RNA synthesis until the morula stage. Ellem and Gwatkin⁷ also found no detectable RNA synthesis at the two-cell stage of development. Recently, using more

TABLE 1 Effect of α -amanitin on development of two-cell mouse embryos 48 h after commencing α -amanitin treatment.

Dose α -amanitin ($\mu\text{g ml}^{-1}$)	Total No. Embryos	No. 2-cell Embryos	No. 3-4 cell Embryos	No. morula- blastocysts	% arrested at 2-cell
0.00	96	16	16	64	17
0.03	47	18	19	10	38
0.3	33	15	16	2	45
1.0	304	220	82	2	71

sensitive techniques, RNA synthesis has been detected as early as the two-cell stage of development⁸⁻¹⁰. We assumed that since RNA synthesis occurs in preimplantation mouse embryos, the enzyme, DNA-dependent RNA polymerase, must be active. We report here the results of *in vivo* and *in vitro* experiments intended to characterise more fully the RNA polymerase present in preimplantation mouse embryos. We used the mushroom toxin, α -amanitin, to determine which forms of RNA polymerase are active and the relative amounts of each form which are present in preimplantation mouse embryos.

In an earlier study¹¹ we reported the existence of three forms of DNA-dependent RNA polymerase in the nuclei of adult mouse liver. It was determined that two of the forms, IA and IB, were resistant to an α -amanitin concentration of $0.3 \mu\text{g ml}^{-1}$ whereas form II was completely inhibited by this amount of toxin. These findings are in general agreement with those of Roeder and Rutter¹² in which it was further determined that form I polymerase is found primarily in the nucleolus and synthesises ribosomal-like RNA while form II is found in the nucleoplasm and synthesises a DNA-like RNA. We thought it should be possible, then, to ascertain whether RNA polymerase form II is active in synthesising mRNA in preimplantation mouse embryos.

A first set of experiments was designed to test the *in vivo* effects of α -amanitin. Preimplantation mouse embryos at the two-cell stage of development were collected from CF1 female mice (Carworth Labs, Inc., Portage, Michigan) after superovulation with 5 IU pregnant mare serum (PMS), (Organon) followed 48 h later by 5 IU human chorionic gonadotropin (HCG), (Nutritional Biochemical). The embryos were collected and washed in Whitten and Biggers medium¹³ and cultured in microdrops of medium (containing one or two embryos) under oil which had been preequilibrated with the medium. The cultures were maintained at 37°C in an atmosphere of 5% CO_2 , 5% O_2 , 90% N_2 . When α -amanitin was used as an inhibitor, it was added to the medium in which the embryos were collected, and was present throughout all further manipulation including equilibration of the oil. The embryos were scanned visually for stage of development 48 h after being introduced into microdrop culture. The results of these experiments are shown in Table 1. It is seen that 17% of the normal embryos failed to develop past the two-cell stage under our culture conditions. The effect of α -amanitin was to arrest development at the two-cell stage with $1 \mu\text{g ml}^{-1}$ of α -amanitin arresting 71% of the embryos

tested. It is interesting to note that although 67% of the untreated embryos developed to the morula or blastocyst stages less than 1% of the embryos which had been treated with $1 \mu\text{g ml}^{-1}$ of α -amanitin developed to the morula or blastocyst stages.

From the *in vivo* experiments reported above it seems that RNA polymerase is indeed active as early as the two-cell stage of development in the mouse. Exactly which of the multiple RNA polymerases is inhibited remains an open question. Although it is generally agreed that *in vitro* only the nucleoplasmic form of the enzyme is inhibited, Tata *et al.*¹⁴ have shown that *in vivo* both the nucleoplasmic and nucleolar polymerases may be inhibited.

For *in vitro* experiments with solubilised enzyme, CF1 mice were superovulated as described earlier and blastocysts collected 86-92 h after HCG injection. The embryos were treated with Pronase to remove the zona pellucida, washed three times in Whitten and Biggers medium, washed once in 0.01 M Tris pH 7.4, 0.5% BSA, and taken up in $10 \mu\text{l}$ of the same buffer. The embryos were frozen and thawed three times in liquid nitrogen. $2.5 \mu\text{l}$ of $2.75 \text{ M } (\text{NH}_4)_2\text{SO}_4$ (pH 7.9) were then added to bring the $(\text{NH}_4)_2\text{SO}_4$ concentration to 0.55 M. $25 \mu\text{l}$ of TGMED (0.05 M Tris-HCl, pH 7.9; 25% glycerol; 5 mM MgCl_2 ; 0.1 mM EDTA; 0.5 mM dithiothreitol) or TGMED containing the desired concentration of α -amanitin were then added followed by $37.5 \mu\text{l}$ of assay mixture. The final reaction mixture ($75 \mu\text{l}$) contained: 36 μg calf thymus DNA, 50 mM Tris-HCl, pH 7.9, 2 mM MnCl_2 , 1.6 mM MgCl_2 , 6 mM NaF, 8 mM KCl, 0.5 mg ml^{-1} BSA, 0.3 mM spermine, 0.5 mM dithiothreitol, 0.6 mM ATP, 0.6 mM GTP, 0.6 mM CTP, 0.004 mM ^3H -UTP (New England Nuclear, specific activity 26.9 Ci mmol^{-1}), 3 μg pyruvate kinase (Sigma, 320 units mg^{-1}), 4 mM phosphoenolpyruvate, 0.09 M $(\text{NH}_4)_2\text{SO}_4$, 8.3% glycerol, 0.03 mM EDTA. The reaction mixture was incubated at 37°C for 40 min and the amount of incorporated ^3H -UMP determined by the method of Litman¹⁵.

The RNA polymerase activity of blastocysts in the presence and absence of $1.1 \mu\text{g ml}^{-1}$ α -amanitin is shown in Table 2. We show data from two experiments, B12 and B13. The average values reported as ^3H -UMP incorporated per embryo per min ($\text{pmol} \times 10^{-5}$) are 2.70 ± 0.42 without α -amanitin and 1.83 ± 0.29 with α -amanitin. This corresponds to 32% inhibition of enzyme activity by $1.1 \mu\text{g ml}^{-1}$ α -amanitin. The average values for experiment B12 alone are 3.09 without α -amanitin and 2.01 with α -amanitin, whereas the average values for experiment B13 alone are 2.44 without α -amanitin and 1.57 with α -amanitin. This corresponds to 35% inhibition in experiment B12 and 36% inhibition in experiment B13. We have found that these types of experiments are always internally consistent, but absolute values may vary from one experiment to another, as illustrated by the data for experiments B12 and B13. For this reason our experiments always include several samples without α -amanitin as a control.

Figure 1 shows the inhibition of mouse blastocyst RNA polymerases by varying doses of α -amanitin. The assays were all performed at high ionic strength, which favours the detection of form II RNA polymerase. When these experiments were repeated at low ionic strength, the results shown in Table 3 were obtained. It is seen that the blastocyst enzymes are inhibited 6% at low ionic strength compared

TABLE 2 Effect of α -amanitin on RNA polymerase activity from mouse blastocysts

Experiment No.	No. embryos	α -amanitin ($1.1 \mu\text{g ml}^{-1}$)	($\text{pmol} \times 10^{-5}$) ^3H -UMP incorporated per embryo per min
B12	100	—	2.96
B12	100	—	3.22
B12	100	+	1.97
B12	100	+	1.80
B12	100	+	2.26
B13	100	—	2.22
B13	100	—	2.75
B13	134	—	2.34
B13	100	+	1.57
B13	100	+	1.57

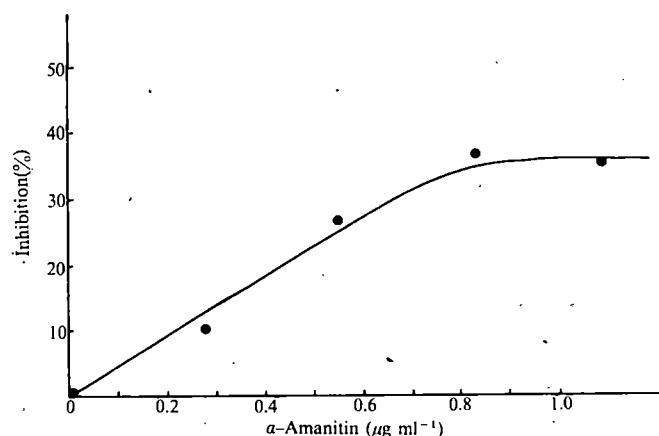


FIG. 1 Inhibition of RNA polymerase activity against concentration of α -amanitin for mouse blastocyst enzymes.

with a maximum of 35% at high ionic strength. In contrast, the adult fraction 4 (F4) enzymes showed 50% maximal inhibition at low ionic strength and 82% maximal inhibition at high ionic strength. We also found that a concentration of 0.30 mg ml^{-1} causes maximal inhibition of F4 and form II adult enzymes, while a concentration of $0.8 \text{ } \mu\text{g ml}^{-1}$ of α -amanitin was necessary for maximal inhibition of the embryonic enzymes (compare Fig. 1 with Table 3). Therefore, it seems that the blastocyst enzymes are less sensitive to inhibition by α -amanitin than are the adult liver enzymes.

There are several possible explanations for these observations. First, it is possible that differences in the solubilisation process for the liver and embryonic polymerases may alter their properties. A second possibility which may effect the α -amanitin sensitivity of the enzymes is the amount of endogenous template present before the addition of α -amanitin. In the embryo experiments no attempt was made to remove the endogenous template while there is very little or no endogenous template present in the solubilised liver enzyme preparation (unpublished observation).

The most attractive possibility is that the difference in maximal inhibition of total RNA polymerase solubilised from blastocysts (35%) and liver nuclei (82%) may indicate that the relative proportions of form I and form II polymerases are different in adult and embryonic tissues and that more form I is present (relative to form II) in blastocysts than in adult liver nuclei. This, as well as the *in vivo* results, represents presumptive evidence for the direct involvement of the various forms of RNA polymerase in the regulation of RNA synthesis in preimplantation mouse embryos.

The question of whether the number of RNA polymerase molecules per cell correlates with the amount of RNA syn-

thesised at any given stage in embryonic development is controversial. Siracusa¹⁶ has reported in a preliminary note that there is no such correlation in mouse embryos from the fertilised egg to the blastocyst stage. Roeder *et al.*¹⁷ also found no such correlation in *Xenopus laevis*, but did find a correlation between the rate of RNA synthesis and the levels of RNA polymerase per cell in the sea urchin¹⁸.

The control of RNA synthesis, however, may be more dependent on the types of RNA polymerase present in early embryos, than on the total amount of enzyme present. The former could specifically control which RNA species were synthesised at any given time, whereas the latter would at best be a reflection of total RNA synthesis. We have shown that at least two distinct forms of RNA polymerase activity, based on their inhibition by α -amanitin, are present in preimplantation mouse embryos. We are currently trying to show which types of RNA are synthesised by the different forms of the enzyme at various stages in preimplantation mouse development.

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TABLE 3 Comparison of effect of α -amanitin on embryonic and adult enzymes at high and low ionic strength.

Enzyme* preparation	α -amanitin ($\mu\text{g ml}^{-1}$)	(NH_4) ₂ SO ₄ (M)	% inhibition of control
F4	0.30	0.01	50
F4	0.30	0.04	73
F4	0.30	0.09	82
IA	0.30	0.04	0
IB	0.30	0.05	0
II	0.01	0.10	72
II	0.30	0.10	100
blastocyst	1.1	0.03	6
blastocyst	1.1	0.09	35

* F4, is the mixture of RNA polymerases from adult mouse liver directly before DEAE-Sephadex chromatography; Forms IA, IB and II are the isolated enzyme forms after DEAE-Sephadex chromatography (see Versteegh and Warner¹¹). Blastocyst enzyme is as described in the text.

Bacteriophage of *Halobacterium salinarum*

Halobacterium salinarum is a member of the Halobacteria, a group of obligate, extremely halophilic organisms requiring at least 15% NaCl in their growth media. No bacteriophage has previously been found in association with members of this group.

H. salinarum strain 1 was cultivated as described by Dundas *et al.*¹. During an investigation of flagella from *H. salinarum*, phage particles were observed in some crudely purified flagellar preparations. Phage-containing preparations did not give rise to plaques when plated with exponentially growing *H. salinarum* cells. Attempts at ultraviolet induction of *H. salinarum* cultures resulted in normal death curves

without any concomitant phage production. Maintenance of batch cultures, started from isolated *H. salinarium* colonies, in the logarithmic phase of growth, by means of serial transfer of cultures in late log phase to fresh media, resulted in eventual lysis of the cultures. Lysis may occur after the first transfer or be delayed for more than six transfers.

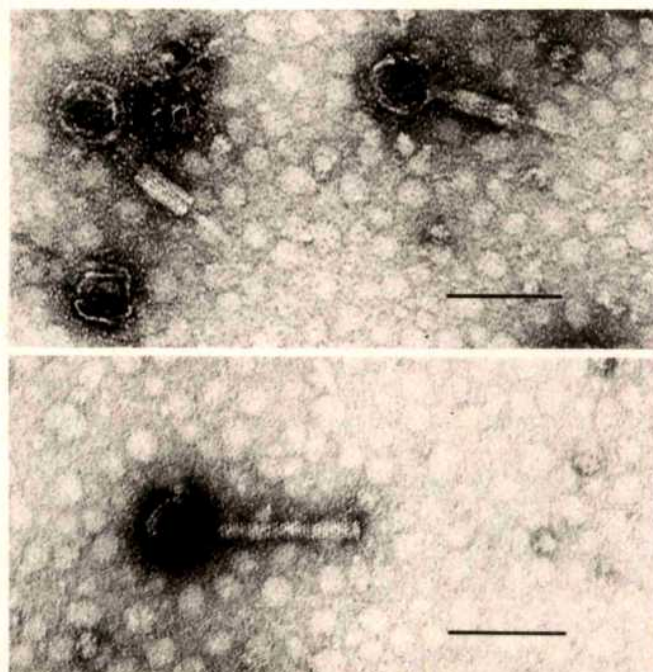


FIG. 1 Electron micrographs of preparations from *H. salinarium* culture lysates. All preparations were negatively stained with uracyl acetate. The scale bar is 0.1 μ m.

In these experiments the cultures initially exhibited normal generation times of 6.1 to 6.7 h. Before lysis, culture generation time increased to more than 10 h. At this point cultures would lyse in 24–48 h after entering late logarithmic growth phase. Lysis took place both in cultures stored without aeration at room temperature and in cultures incubated with aeration at 37°C. Normal cultures (generation time 6–7 h) will not lyse when incubated for more than a week with or without aeration, at 37°C or at room temperature.

All culture lysates produced in this manner contained phage particles. (Fig. 1). Phage particles have not yet been found by routine electron microscopic examination of normal cultures at any stage of growth. The phage particles have an isometric polyhedral head and a contractile tail. The dimensions of the particles are quite uniform.

The existence of bacteriophage for *Halobacterium* opens the existing possibility of further exploration of the genetic basis of extreme halophilism.

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Further observations on the structure of the lipid-containing bacteriophage PM2

RECENT work on the proteins of bacteriophage PM2 have led us to a model for the structure of this virus, proposed in this communication. Experimental results have enabled us to assign each of the four proteins¹ to morphological structures of the virus revealed by electron microscopic and structural studies. Packing considerations indicate that protein II must form the outer protein shell². Both proteins I and II are on the outside of the phage particle as demonstrated by labelling with radioactive iodine after lactoperoxidase treatment³ by the method of Phillips and Morrison^{4,5}.

In agreement with these results we have now shown that protein I can be selectively removed by digestion with bromelain, a proteolytic enzyme. Furthermore protein II can be labelled with ³⁵S-sulphanilic acid-diazonium salt by the method of Berg⁶ (Fig. 1). We have also isolated a nucleocapsid core by dissociation of the virion in 4.5 M urea. This core, which has an approximate $S_{20,w} = 133$ and which has been identified by electron microscopy as a well-organised icosahedrally-shaped core structure, contains all protein III and IV, as well as the viral DNA, but no phospholipids and

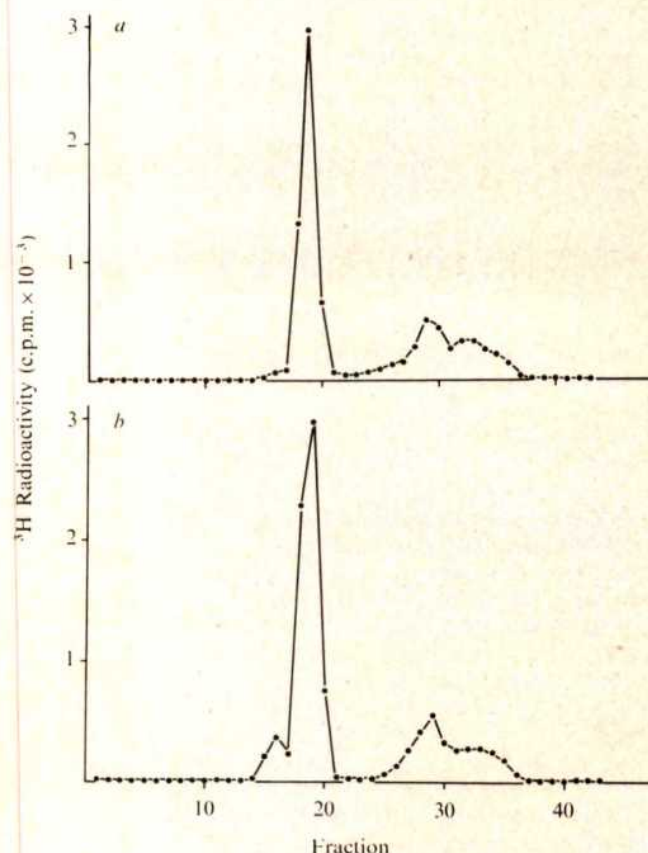


FIG. 1 a, Digestion of protein I by bromelain ³H-amino acid-labelled PM2 virus was added to 1 ml of 0.01 M sodium phosphate buffer (pH 7.0), containing 0.1 M NaCl, 0.001 M CaCl₂. After the addition of 1 mg bromelain, the mixture was incubated at 30°C for 3 h. The ratio of PM2 protein to protease was 0.1. At the end of the incubation, the mixture was chilled on ice. The protease was separated from PM2 on a sucrose step gradient, containing 20%, 40%, and 60% sucrose layers. Centrifugation was carried out at 120,000g for 1 h. After centrifugation, the virus band was collected and subjected to SDS-gel electrophoresis¹. In a control experiment (b) the virus was prepared for gel electrophoresis by the same procedures as described above, without digestion with the proteolytic enzyme. In both cases, after gel electrophoresis the gels were frozen and sliced into 0.9 mm fractions. The slices were placed in glass vials containing 8 ml toluene-PPO-POPOP-mixture and 3% Protosol. Vials were incubated with shaking overnight at 37°C and counted in a Packard liquid scintillation counter.

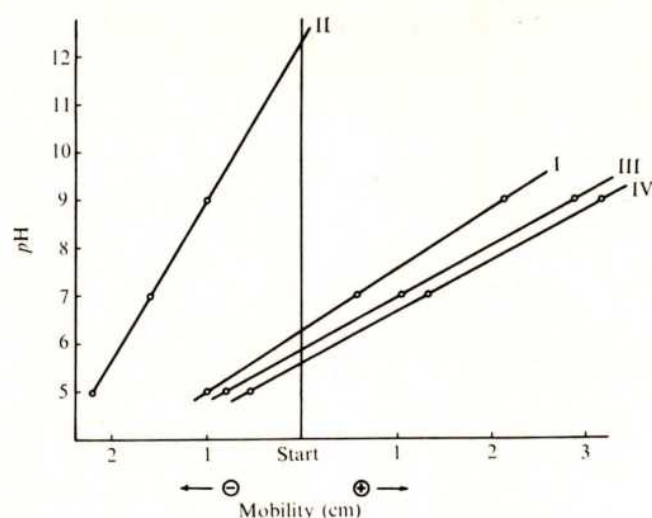


Fig. 2 Estimation of the isoelectric points of the individual PM2 proteins by cellulose-acetate electrophoresis. Before electrophoresis, cellulose-acetate sheets (4×17 cm) were equilibrated for 10 min in separation buffers (0.5 M Tris acetate pH 5, 7, or 9; 6 M urea, 0.02 M 2-mercaptoethanol). Protein solutions (5–20 μ l) (10–20 mg ml⁻¹ PM2 virus) were applied to the gel with a polyethylene capillary tip. Electrophoresis at the different pH values were carried out in a moist chamber on a refrigerated Teflon-coated surface for 90 min at constant voltage (40 V cm⁻¹). After electrophoresis, the protein was stained with a 0.25% (w/v) amido black 10B solution which contained methanol, water, acetic acid (45:45:10 (v/v)). The solvent (without amido black) was used for destaining. The running distances of the individual PM2 polypeptides and reference proteins (not shown) were plotted against the different pH values. To determine the isoelectric points these mobilities were extrapolated to zero mobility.

no protein I and II. Thus protein II, the major viral protein, forms the outer icosahedral shell; proteins III, IV and the DNA the inner icosahedral shell of the nucleocapsid, and the phospholipid bilayer is sandwiched between these two shells. Protein III probably forms the protein bridges⁷ which connect the inner and outer shells through the bilayer. From preliminary electron microscopic studies and from experiments showing that the phage cannot bind to its host cell after digestion of protein I, we imagine that protein I must form the outer spikes found at the vertices of the icosahedron.

We also measured the isoelectric points of the four viral proteins. These were determined on disrupted virus particles by electrophoresis on cellulose acetate strips at various pH values. The mobilities were extrapolated to zero mobility, a method preferable to isoelectric focusing in which the virus proteins precipitate near the isoelectric point. Whereas proteins I, III, and IV have isoelectric points at slightly less than neutrality (pH 6.2, 5.8, and 5.5, respectively), the isoelectric point of protein II is 12.3 (Fig. 2). Thus whereas proteins I, III, and IV are slightly negatively charged at pH 7.0, protein II is strongly positively charged.

From previous studies, PM2 contains 12.6–14.0% of phospholipid, of which 64% is phosphatidylglycerol (PG) and 27% is phosphatidylethanolamine (PE)^{8,9}. These ratios of phospholipids are essentially the inverse of those of the host cell (23% PG and 75% PE)⁸. As PG is negatively charged and PE is neutral at neutral pH values, we propose that electrostatic interactions between protein II and PG must play a major role in the structural integrity as well as in the assembly of phage PM2. We predict that there must be a very strong asymmetry in the bilayer, with PG preferentially located in the outer lamella of the bilayer. From calculations of the number of PG molecules and the amount of positive charge due to protein II there is more than enough positive charge to neutralise the charge on PG, as well as

the excess negative charge of proteins I, III, and IV. Experiments to test the hypothesis of bilayer asymmetry, as well as experiments to determine the electrophoretic mobility of the virion are now in progress. Bilayer asymmetry has already been demonstrated for the erythrocyte membrane^{10,11}.

From a study of the origin of the viral phospholipids it is clear that the difference in viral phospholipid composition from that of the host cell must be due to selective processes occurring during virus assembly¹². There are active selective processes (biochemical processes) involving phospholipid synthesis and hydrolysis¹². There must also be passive (physical) selective processes and we propose that the electrostatic interaction of protein II with PG must play a major part in this latter process. Our present studies on PM2 structure, synthesis, and assembly should provide valuable information on protein-protein and protein-lipid interactions in the formation of one type of natural bilayer.

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Fucosylglycolipids in cells transformed by a temperature-sensitive mutant of murine sarcoma virus

We have reported that fucosylglycolipid metabolism is markedly altered in cells transformed by oncornavirus¹. Transformed cells exhibit a sharp decrease in radioactive fucose incorporation into a chromatographically less mobile fucosylglycolipid, with a corresponding increase in radioactivity in chromatographically more mobile fucosylglycolipids. We describe here further studies of fucosylglycolipid metabolism using normal rat kidney (NRK) cells transformed by a cold-sensitive mutant of murine sarcoma virus (MSV)². The cell line, designated NRK (MSV-1b), is cold sensitive for maintenance of the transformed phenotype. The cells express the transformed phenotype at the permissive temperature (39° C) but appear phenotypically normal at the non-permissive temperature (33° C). Cells transformed by this mutant are non-producer cells, but contain the MSV genome in a rescuable form. Using these cold-sensitive transformants, we were able to demonstrate, in a temporal fashion, a relationship between altered fucosylglycolipid metabolism and the development of the transformed phenotype.

TABLE 1 Incorporation of ^{14}C -fucose into glycolipids of normal (NRK), NRK (MSV-1b) at non-permissive and permissive temperatures, and transformed NRK (MSV-MLV) cell lines

Fucosylglyco- sphingolipid	1 NRK (normal)		2 NRK (MSV-1b) at 33°C (non-permissive)		3 NRK (MSV-1b) at 39°C (permissive)		4 NRK (MSV-MLV) (transformed)	
	Compound	% c.p.m. per mg protein	Compound	% c.p.m. per mg protein	Compound	% c.p.m. per mg protein	Compound	% c.p.m. per mg protein
FucCer I	1.6	121	1.3	100	1.5	91	3.8	316
FucCer II	2.3	174	4.0	308	4.6	280	5.6	466
FucCer III	24.0	1814	27.6	2127	65.8	3999	80.5	6700
FucCer IV	72.0	5443	67.0	5163	28.1	1702	10.1	832

Culture conditions and thin-layer chromatography are described in Fig. 1. Compounds were located by autoradiography on Kodak No-screen X-ray films, scraped and quantitated by scintillation spectroscopy. Protein determination was carried out by the Lowry method. Permissive temperature (that is, phenotypically transformed) was 39° C; non-permissive temperature (that is, phenotypically normal) was 33° C.

All cells were grown in glass bottles in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum (v/v). Cell monolayers were washed three times with phosphate-buffered saline (PBS), removed by scraping into PBS and sedimented to a pellet. The pellet was washed with PBS and the lipid was extracted¹. The fucosylglycolipids were characterised as previously described¹, with the additional observation that pronase treatment³ had no effect on the chromatographic behavior of the fucosylglycolipids.

To investigate fucosylglycolipid metabolism at permissive and non-permissive temperatures, cells were seeded in medium supplemented with 0.5 $\mu\text{Ci ml}^{-1}$ of U- ^{14}C -L-fucose (133 mCi mmol⁻¹, Amersham/Searle, Arlington Heights, Illinois) and were grown at either 33° C or 39° C until they reached confluence (~ 72 h). On a protein basis, comparable amounts of radioactivity were incorporated into the fucosylglycolipids of uninfected NRK cells, the NRK (MSV-1b) cells grown at the permissive or non-permissive temperature, and into NRK cells transformed by the Moloney leukaemia-sarcoma virus complex (NRK (MSV-MLV), Table 1). Approximately equal amounts of radioactivity were chroma-

tographed (Fig. 1), and the major radioactive areas were excised from the plate and counted (Table 1). Consistent with the previous study¹ of other oncornavirus-transformed cell lines, there was a considerable alteration in the pattern of fucose labelling of glycolipids of the virus-producing, transformed NRK (MSV-MLV) cells (Fig. 1, lane 4; Table 1, column 4) compared with uninfected NRK cells (Fig. 1, lane 1; Table 1, column 1). NRK (MSV-MLV) cells exhibited a marked decrease in the amount of the chromatographically less mobile fucosylglycolipid (FucCer IV) with a concomitant increase in the chromatographically more mobile FucCer III. The fucosylglycolipid patterns of uninfected NRK cells and of NRK (MSV-MLV) cells were the same in cells cultured at 33° C or 39° C. Examination of the fucosylglycolipid pattern of cold-sensitive NRK (MSV-1b) cells grown at permissive temperature (Fig. 1, lane 3; Table 1, column 3) revealed a pattern which resembled the transformed NRK (MSV-MLV) cells. However, the inhibition of synthesis of the chromatographically less mobile component (FucCer IV) was not as marked in the NRK (MSV-1b) cells grown at permissive temperature as in the NRK (MSV-MLV) cells. In this regard, other measurements at permissive temperature (for example, sugar transport and saturation density)⁴ suggest less expression of viral transforming function in cells transformed by the cold-sensitive mutant compared with cells transformed by the parental strain of MSV-MLV. Examination of the fucosylglycolipid pattern of NRK (MSV-1b) cells grown at the non-permissive temperature (Fig. 1, lane 2; Table 1, column 2) demonstrated a striking resemblance to the pattern observed with normal cells.

We next measured the effect of temperature shift of NRK (MSV-1b) cells on fucosylglycolipid synthesis. Based on the results of the above experiments, reciprocal temperature-shift studies were performed to examine, in a temporal fashion, the relationship between fucosylglycolipid metabolism and the appearance of the transformed phenotype. Twenty-four hours after shifting to the permissive temperature, there were no marked morphological changes, and the fucosylglycolipid profile was comparable with cultures maintained at nonpermissive temperature (Fig. 2a and b, 24 h). After 48 h most cells were morphologically transformed and the fucosylglycolipid pattern of these cultures resembled that of cultures maintained continuously at the permissive temperature rather than of those maintained continuously at the non-permissive temperature (Fig. 2, a compared with b and c, 48 h). There was an increase in FucCer III, and decreases in FucCer I, II and IV. After 72 h the cultures shifted to the permissive temperature were essentially all transformed and the fucosylglycolipid pattern was the same as that of cultures maintained continuously at the permissive temperature (Fig. 2b and c, 72 h). On the other hand, 24 h after the reciprocal shift from permissive to non-permissive temperature, both the morphology and fucosylglycolipid pattern (Fig. 2d, 24

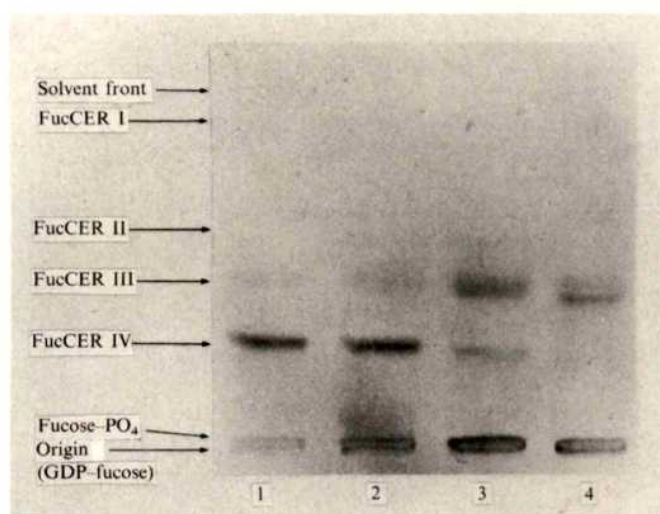


Fig. 1 Autoradiogram of the ^{14}C -fucose-labelled glycolipids from: (1) NRK cells, (2) NRK (MSV-1b) cells grown at 33° C (non-permissive temperature), (3) NRK (MSV-1b) cells grown at 39° C (permissive temperature) and (4) NRK (MSV-MLV) transformed cells. Culture conditions and lipid extraction are described in the text. Lipids were chromatographed on hard silica gel plates 'Q5' (Quantum Industries, New Jersey) in 2-propanol-ammonia (specific gravity 0.88)-water (7:2:1, by volume). Tentative identification of GDP-fucose and fucose- PO_4 is based on chromatographic similarities to several sugar-nucleotides and sugar-phosphates in ethanol-1 M ammonium acetate (7:3, v/v; pH 7.5). The radioactivity of these components has been excluded from the calculations in Table 1.

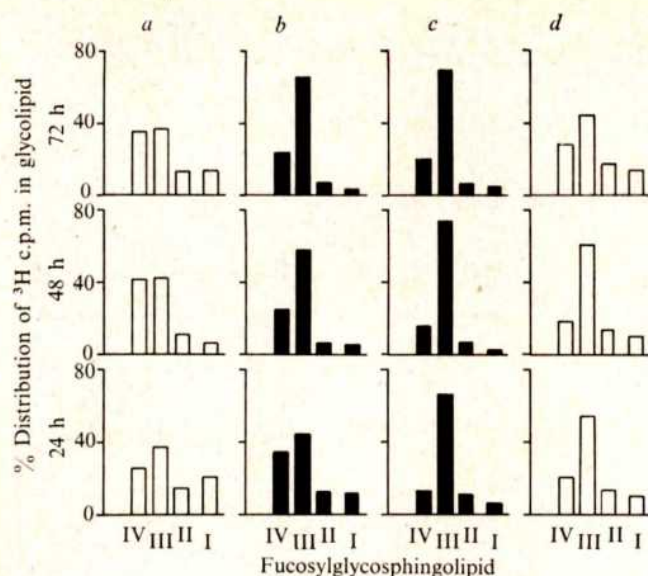


Fig. 2 Profiles of ^3H -fucose-labelled glycolipids after reciprocal temperature shifts. NRK (MSV-1b) cells were grown at either 39°C (permissive (P) temperature) or 33°C (non-permissive (NP) temperature) for 72 h before addition of isotopically-labelled fucose to minimise possible cell density-dependent effects on glycolipid synthesis⁵. The medium was then removed and fresh medium containing $1\text{-}^3\text{H-L-fucose}$ ($5\text{ }\mu\text{Ci ml}^{-1}$, 1.4 Ci mmol^{-1} , Amersham/Searle) was added. Half the cultures grown at 33°C (NP) were shifted to 39°C (NP \rightarrow P), while half of those at 39°C (P) were shifted to 33°C (P \rightarrow NP). At successive 24 h intervals thereafter, the cultures were examined morphologically and the fucosylglycolipids were analysed. Chromatography was carried out as in Fig. 1. The thin-layer plates were scraped and the major fucosylglycolipids (see Fig. 1) were counted by scintillation spectrometry. a, NP; b, NP \rightarrow P; c, P; d, P \rightarrow NP.

h) were comparable with cultures maintained at the permissive temperature. After 48 h many of the cells appeared morphologically normal and the fucosylglycolipid pattern resembled that of cultures maintained at the non-permissive temperature (that is, decrease in FucCer III, increase in FucCer I, II and IV). After 72 h essentially all the cells shifted to the non-permissive temperature appeared morphologically normal and the fucosylglycolipid pattern was comparable with that of cultures maintained continuously at the non-permissive temperature (Fig. 2a, 72 h) rather than that of cells maintained at the permissive temperature (Fig. 2c, 72 h).

These data show that alterations in fucosylglycolipid metabolism are related directly to the expression of the transformed state, and are not simply the result of MSV infection. The fucosylglycolipid pattern of NRK (MSV-1b) cells grown continuously at permissive temperature closely resembles the fucosylglycolipid pattern of cells transformed by the wild-type parent strain of MSV-MLV. In a parallel fashion, the fucosylglycolipid pattern of NRK (MSV-1b) cells grown continuously at non-permissive temperature resembles that of normal cells. In contrast, studies of nonfucose glycolipids (that is, gangliosides and their neutral glycolipid precursors) involving hamster cells infected with a temperature-sensitive (ts) transformation mutant of polyoma virus⁵ or chick embryo cells with a ts transformation mutant of Rous sarcoma virus⁸ have revealed similar non-fucose glycolipid patterns at both permissive and non-permissive temperatures. In addition, kinetic studies using the cold-sensitive NRK (MSV-1b) cells in temperature-shift experiments revealed a relationship between the morphology of the transformed cell phenotype and altered fucosylglycolipid synthesis. The relative rapidity of the fucosylglycolipid alterations after temperature-shift as compared with the stable pattern of the non-fucose glycolipids^{3,5}, suggests a unique and dynamic role for fucosylglycolipids in transformation.

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Carbohydrate groups in the major glycoprotein of Rous sarcoma virus

GLYCOPROTEINS and other complex carbohydrates on the cell surfaces have been thought to be involved in many biological interactions¹. Most enveloped viruses contain at least one glycoprotein component¹. In avian sarcoma and leukaemia viruses some of the glycoproteins are located on the surfaces of the particles as projections composed of spikes and knobs²⁻⁴. Although their role is not well understood, the glycoproteins are believed to be involved in the determination of host range specificity, induction of neutralising antibody and interference capacity^{2,5-11}. Rous sarcoma virus contains several glycoproteins¹⁰⁻¹³, and infectious and non-infectious strains of Rous sarcoma virus differ in major glycoprotein components^{14,15}. We report here the results of purification of the major glycoprotein component (g2) of Rous sarcoma virus, its amino acid composition, and the nature of its carbohydrate groups.

The Bryan high-titre strain of Rous sarcoma virus RSV (RAV-1) was grown in type C/O or C/B chick embryo

TABLE 1 Amino acid composition of glycoprotein g2

Amino acid	Mol %
Lys	3.66
His	2.81
Arg	4.47
Asp	11.26
Thr	7.56
Ser	8.47
Glu	11.70
Pro	6.97
Gly	9.33
Ala	5.24
Cys	—
Val	5.36
Met	1.34
Ileu	3.90
Leu	8.33
Tyr	4.51
Phe	5.07

The glycoprotein, with added norleucine standard, was hydrolysed in 6 N HCl for 22 h at 105°C . Amino acid analysis was performed on a Technicon TSM amino acid autoanalyser.

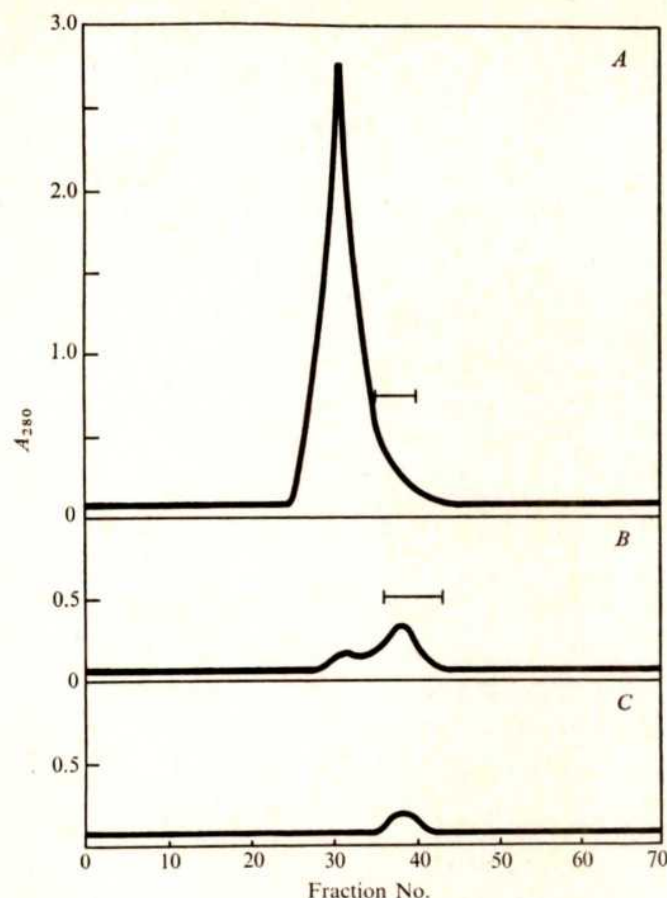


Fig. 1 Chromatography of viral proteins with Bio-Gel P-150. A, The phenol-extracted proteins of RSV(RAV-1) were passed through a column and fractions 35-40 (3.6 ml per fraction) were combined for rechromatography. B, The optical density profile after rechromatography of fractions 35-40 from (A). Fractions 36-43 were combined for further purification. C, Chromatography of the combined fraction from (B). Fractions 36-40 which contain homogeneous g2 glycoprotein by SDS-gel electrophoresis, were combined for the studies of amino acid and sugar compositions.

fibroblasts in an appropriate growth medium¹⁶. Large quantities of virus were isolated and purified from 40-60 l of culture medium by differential centrifugation and isopycnic banding as described before¹⁷. Viral protein was extracted with phenol from a preparation containing about 550 A_{280} units of purified RSV(RAV-1) in the presence of sodium dodecylsulphate (SDS) and EDTA as reported earlier¹⁸. The viral protein was dissolved at 56°C in 0.1 M Tris buffer, pH 8.3 containing 6 M urea and 1% mercaptoethanol. Gel-filtration chromatography was carried out on a column of Bio-Gel P-150 (2.7 × 93 cm) in sodium phosphate buffer, 0.01 M, pH 7.2, and 0.1% mercaptoethanol. To locate glycoprotein g2, aliquots from the collected fractions were electrophoresed in SDS gels¹³ and stained with Coomassie brilliant blue. The glycoprotein was detected in the trailing region, fractions 35-40 (Fig. 1A) of a peak near the void volume. Rechromatography of the trailing region on the same column showed two peaks (Fig. 1B). Rechromatography of fractions 36-43 (Fig. 1B) produced a peak containing only the glycoprotein g2 as analysed in SDS gel electrophoresis (Fig. 2). In a typical run, about 1.8 A_{280} units of the protein were isolated.

Amino acid and sugar compositions (Tables 1 and 2) indicate that g2 is composed of 40% by weight of carbohydrate. A preparation containing 1.8 A_{280} units contained 0.95 mg of peptide and 0.68 mg of carbohydrate. The minimal molecular weight calculated from the results of Tables 1 and 2 is about 12,500. From SDS-gel electrophoresis results, the

TABLE 2 Sugar composition of glycoprotein g2

Sugar	$\mu\text{mol per mg peptide}$
Glucosamine	1.36
Mannose	1.20
Galactose	0.64
Fucose	0.10
Sialic acid	0.40

Hydrolysis conditions were as follows: for glucosamine, 4 N HCl for 6 h at 100°C; for neutral sugars, 2 N trifluoroacetic acid for 2 h at 100°C; for sialic acid, 0.05 N H_2SO_4 for 1 h at 80°C. Amino sugars and neutral sugars were determined by automated assay as described^{19,20}. Electronic expansion of the signal from the colorimeter by means of a linear converter (Dynacon Research and Systems, Pallisades, New York) facilitated determination of 0.001 to 0.02 μmol of amino sugars. Sialic acid was assayed with autoanalytical chromatography (our work to be published) using the thiobarbituric acid reaction²¹ to develop colour after elution of the sample from an anion exchange resin column. Each sugar analysis required about 25 μg of glycoprotein, except that 150 μg was used for the determination of fucose. All sugar measurements were made in the range of 5-15 nmol. No corrections were made for destruction of sugars during acid hydrolysis.

value of 75,000 had been assigned previously¹³. Although radioactive fucose has been shown to incorporate rapidly into glycoproteins of RSV¹⁰, chemical analysis shows a relatively small content of fucose.

Up to 45% of the sugar residues could be removed by digestion with glycosidases. Table 3 shows the release of sugars by α -neuraminidase, β -galactosidase and β -N-acetylhexosaminidase, incubated separately and in combinations. All the sialic acid was released by neuraminidase. β -Galactosidase alone released 25% of the galactose, suggesting that some galactose residues are at terminal positions. After removal of sialic acid most remaining galactose residues were susceptible to galactosidase; about one additional mole was released per mole of sialic acid cleaved. Very little N-acetylglucosamine was released from intact g2 by β -N-acetylhexosaminidase. When neuraminidase and galactosidase were included in the incubation, nearly 50% of the N-acetylglucosamine was released—an amount about equal to the total galactose content of g2.

These results suggest that the structure of the terminal region of the carbohydrate unit consists largely of sequences: sialic acid \rightarrow galactose \rightarrow N-acetylglucosamine \rightarrow , and galactose \rightarrow N-acetylglucosamine \rightarrow . The same sequences have been found in many soluble vertebrate glycoproteins¹. Recently, rabbit interferon was shown to have sialic acid \rightarrow galactose \rightarrow sequence²³. The positions of mannose and the other glucosamine residues in the g2 glycoprotein have yet to be determined. The major glycoprotein described here corresponds to the knob projections in avian RNA tumour viruses², and seems to perform an important function as it may recognise and interact with the receptor sites of

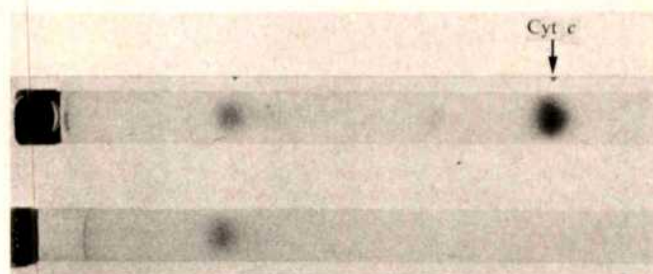


Fig. 2 Polyacrylamide gel electrophoresis of the purified glycoprotein from Bio-Gel P-150 chromatography. Aliquots of the combined fractions from Fig. 1C were electrophoresed alone (bottom) or together with horse heart cytochrome c (top) in SDS gels. The mobility of cytochrome c was about 3.4 times faster than that of the g2 glycoprotein.

TABLE 3 Glycosidase digestions of the g2 glycoprotein.

Sugar measured	Enzyme treatment	μ mol released per mg peptide	% Release
Sialic acid	α -Neuraminidase	0.43	107
Galactose	β -Galactosidase	0.15	23
	β -Galactosidase + α -Neuraminidase	0.59	92
Glucosamine	β -Hexosaminidase	0.05	4
	β -Hexosaminidase + β -Galactosidase + α -Neuraminidase	0.64	47

Neuraminidase (*C. perfringens*) was obtained from Boehringer Mannheim. Digestion was carried out overnight at 37°C in 0.03 M potassium acetate, pH 4.5, containing 0.03% bovine serum albumin (BSA), with an enzyme to substrate ratio of 1:15. Under these conditions, sialic acid was liberated completely from α -acid glycoprotein. Aliquots of digestion mixture were assayed directly by the autoanalytical method (see Table 2). Jack bean β -galactosidase²² was obtained from Dr Y. T. Li. Digestion was carried out at 23°C for 3 d in 0.03 M potassium acetate, pH 4.0, containing 0.03% BSA, a trace of thymol as preservative, and 1.5 enzyme units per 100 μ g of glycoprotein. Digestion was monitored at 24-h intervals by direct application of aliquots of mixture to the autoanalytical system. The enzyme remained active during the entire incubation period. Jack bean β -N-acetylhexosaminidase²² was obtained from Dr Y. T. Li. Digestion was carried out at 23°C for 3 d in 0.06 M sodium citrate, pH 4.0, containing 0.02% BSA, a trace of thymol, and 25 units of enzyme per 100 μ g of glycoprotein. Digestion was monitored at 24-h intervals. The liberated N-acetylglucosamine was deproteinized with Dowex 50, deacetylated with 4 N HCl for 6 h at 100°C, and assayed as free glucosamine using the autoanalytical system. In experiments where combinations of enzymes were used, the incubation conditions for all enzymes were those appropriate for the first listed enzyme.

permissive host cells during the first step of a successful infection. Involvements of carbohydrate moieties during cellular interactions have been hypothesized²⁴, and there is some supporting evidence²⁵. The interplay between cell surface sialic acids and viral neuraminidase is now well understood²⁶. It will be interesting to compare sugar sequences and amino acid compositions of glycoproteins from various strains of RSV. These glycoproteins are now being isolated and characterised.

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Early changes in the kidneys of BALB/c mice infected with lactic dehydrogenase virus

Mice infected with lactic dehydrogenase virus (LDV) have lifelong viraemia and raised plasma enzyme levels¹⁻⁴. In spite of rapid and prolonged virus replication, probably in the macrophages, only minor histological changes in lymphoid organs are reported⁵. Antibodies are produced⁶ and complexes are said to be deposited in the glomerular capillary walls but only minimal glomerulonephritis results^{7,8}.

Electron microscopic studies of liver, spleen and lymph nodes failed to show any changes, but in the kidneys of BALB/c mice there were striking changes as early as 24 h after infection, which persisted for at least 3 months. They were confined to the glomeruli and consisted of cytoplasmic endothelial swelling and darkly staining deposit between endothelial cells and basement membrane. There were no basement membrane changes or foot process adhesions. The electron micrograph (Fig. 1) of a glomerulus from an animal 12 h after infection shows markedly swollen endothelial cells filling the lumen of the glomerular capillaries. A dark exudate is present between many endothelial cells and the basement membranes, which are normal.

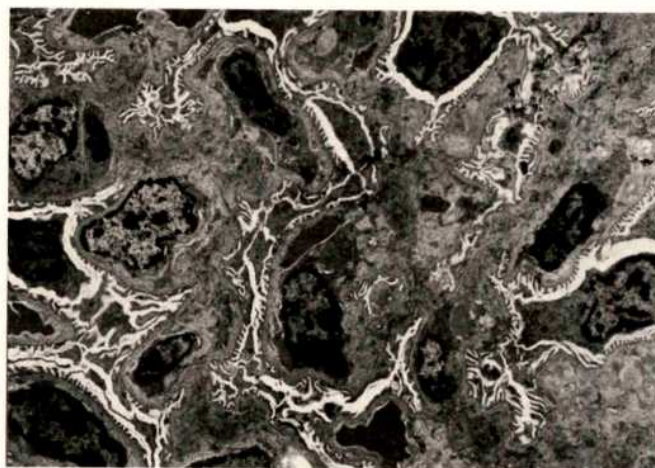


FIG. 1 Electron micrograph of a section of a glomerulus 12h after infection with LDV (X1,880).

TABLE 1 Effect of nephrectomy on replication of lactic dehydrogenase virus.

	Blood virus titre (log ₁₀ ID ₅₀ ml ⁻¹)	
	4 h and 24 h after infection	
Intact	3.5	8.0
Nephrectomised 2 h after infection		3.0
Nephrectomised 4 h after infection		6.0

All the mice were injected intravenously with the same dose of virus and the figures given are the means of three titrations using two mice at each 10-fold dilution.

The early changes in the endothelial cells indicate that the virus might be replicating in them. To investigate this, the effect of bilateral nephrectomy on the level of infectivity of LDV 24 h after infection was measured. As shown in Table 1, total nephrectomy 2 h after infection reduced the level of viraemia at 24 h. The nephrectomised mice seem normal after the operation but by 24 h they were very ill. It was therefore likely that the reduced level of viraemia in the nephrectomised mice was due to toxæmia rather than removal of a site of virus production. This view was supported by the observation that nephrectomy had less effect on virus levels if delayed for 4 h after infection, although no new virus enters the blood until 5 to 6 h after infection.

Unlike other authors^{7,8} we have not found glomerular immune complex deposition in LDV-infected BALB/c mice in the first 3 months, so the changes in the kidneys seen by electron microscopy cannot be related to these deposits. It is possible that the glomerular lesions described here prevent the deposition of immune complexes in the glomeruli. Infection with LDV reduces the severity of spontaneous glomerulonephritis in New Zealand mice⁹. This may be due to reduced levels of antibody⁹ but perhaps the changes produced in the glomerular capillary endothelium by the virus play some part. It would be interesting to know how infection with LDV would affect the development of nephritis resulting from chronic infection with lymphocytic choriomeningitis virus.

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Reticulocyte DNA polymerase

DNA polymerase has been isolated from the cytoplasm of several mammalian tissues as well as several cell lines in culture¹⁻⁸. This has been puzzling because DNA replication and repair are processes generally believed to be restricted to the nucleus, where chromatin is located. It has been suggested that cytoplasmic DNA polymerase is newly synthesised, not yet having been transported to the nucleus⁹ or that it could have leaked from the nucleus during cell disruption^{10,11}. Investigation of possible functions of a DNA polymerase in the cytoplasm have been inhibited by the possibility that the cytoplasmic location of this enzyme is either artefactual or trivial. To demonstrate that this is not so, we have investigated the presence of the enzyme in the reticulocyte, which is an anucleate cell. We wish to report here the purification and characterisation of a DNA polymerase from rabbit reticulocytes, and compare its properties with those of the DNA polymerase purified from the cytoplasm of immature erythroblasts of bone marrow, the precursor of the reticulocyte.

Reticulocytosis was induced by the procedure of Borsook *et al.*¹² New Zealand rabbits (4-6 pounds) were given four daily injections of 1.0 ml of 2.5% neutralised phenylhydrazine. Three days later they were bled by cardiac puncture. Whole blood contained more than 80% reticulocytes.

Washed reticulocytes were prepared by repeated suspension in 130 mM NaCl, 5.0 mM KCl and 7.4 mM MgCl₂, centrifugation at 2,000 *g*, and removal of the buffy coat (nucleated cells and platelets). Washed reticulocyte preparations usually contained less than 0.5% nucleated cells, of which approximately 50% were immature red cells. Comparison of enzyme preparations from washed reticulocytes and from buffy coat cells indicated that enzyme activity is proportional to total cell number and not to the number of nucleated cells.

The packed reticulocytes from 400 ml of whole blood were lysed by addition of 250 ml lysis buffer (10 mM Tris-HCl, pH 7.4, 15 mM KCl and 5 mM 2-mercaptoethanol) followed by gentle stirring for 20 min at 0° C. Cell debris was removed by centrifugation at 30,000*g* for 15 min. The supernatant was made 5.0 mM in MgCl₂ and 0.5 M in KCl and the polysome fraction was removed by centrifugation at 78,000*g* for 120 min. The supernatant was made 40% saturated in (NH₄)₂SO₄ by addition of solid (NH₄)₂SO₄ over 2 h. The (NH₄)₂SO₄ precipitate was collected by centrifugation at 30,000*g* for 15 min, suspended in TSED (50 mM Tris-HCl, pH 7.8, 1.0 mM dithiothreitol, 1.0 mM EDTA and 0.25 M sucrose), and dialysed against 1 l of TSED for 3 h with one change of buffer. The dialysed extract was applied to a phosphocellulose column (2.3 × 18 cm), previously equilibrated with TSED, and washed with the same buffer. A linear gradient of 0 to 1.0 M KCl in TSED with a combined volume of 300 ml was applied and 3 ml fractions were collected. DNA polymerase activity was eluted at 0.25 M KCl. The phosphocellulose fractions with activity were pooled and dialysed against 1 l of TGED (50 mM Tris-HCl, pH 7.8, 1.0 mM dithiothreitol, 1.0 mM EDTA, 25% glycerol) for 4 h with one change of buffer. The dialysed enzyme was applied to a DEAE-Sephadex A-50 column (1.8 × 9 cm), previously equilibrated with TGED, and washed with the same buffer. DNA polymerase was eluted stepwise with TGED containing 0.25 M KCl. A summary of the enzyme purification is shown in Table 1. As haem has been found to be a potent inhibitor of the enzyme, it is possible that the low activity of the crude enzyme fractions is due to inhibition by haem. The overall purification obtained was 2,400-fold with a yield of 73%.

In contrast to the cytoplasmic DNA polymerase of erythroblasts which is associated with the microsomes and

TABLE 1 Purification of DNA polymerase

Step	Total Activity (U)	Protein (mg)	Specific Activity (U mg ⁻¹ protein)	Fold-purification	% Yield
30,000g supernatant	706	28,125	.025	—	—
78,000g supernatant	585	24,750	.024	—	—
40% (NH ₄) ₂ SO ₄ Precipitate	1,000	1,280	.788	31	(100)
Phosphocellulose chromatography	939	23.4	40.9	1,600	(94)
DEAE Sephadex chromatography	727	12.2	60.6	2,400	(73)

The standard assay mixture contained in a final volume of 0.25 ml: 40 mM HEPES (N-2-hydroxyethylpiperazine-N'-2'-ethanesulphonic acid) buffer, pH 7.0, 0.4 mM MnCl₂, 60 mM (NH₄)₂SO₄, 0.16 mM each dATP, dGTP and dCTP, 0.004 mM ³H-dTTP, 2,000 μCi μmol, 20 μg activated calf thymus DNA and 5–10 μg enzyme. The reaction mixture was incubated for 15 min at 37°C and stopped by the addition of 2 ml of cold 5% trichloroacetic acid. The precipitate was collected on a glass fibre filter (Whatman GF/C) and washed with trichloroacetic acid and ethanol. The filter was dried and counted in a liquid scintillation counter. Activated calf thymus DNA was prepared as described by Aposhian and Kornberg¹³. One unit of activity is defined as the amount of enzyme which incorporates one picomole of dTMP in 15 min of incubation at 37°C.

is only solubilised in the presence of high salt concentrations, most of the reticulocyte enzyme is present in the cytosol even at low ionic strength.

The requirements for the synthesis of DNA are shown in Table 2. There is an absolute requirement for a template and a divalent cation. The rate of DNA synthesis is stimulated about three-fold by monovalent cation and about 10%

TABLE 2 Requirements for DNA synthesis

Reaction conditions	³ H-dTMP incorporated (pmol)	% Control
Complete	13.3	100
—dithiothreitol	12.0	90
—divalent cation	0.5	4
—monovalent cation	4.0	30
—template	0.4	3
—enzyme	—	—
—dATP	3.9	30
—dATP, dCTP, dGTP	2.3	17

Incubation conditions were the same as described in Table 1 except that the individual components were omitted as indicated.

by dithiothreitol. All four deoxyribonucleoside triphosphates are required for optimal activity and in the absence of one or more deoxyribonucleoside triphosphates there is a substantial decrease in the rate of dTMP incorporation. This has also been observed with other mammalian DNA polymerases when one (or more) deoxyribonucleoside triphosphate was omitted^{6,14}.

The divalent cation requirement can be satisfied by either Mn²⁺ or Mg²⁺, although at different concentrations (Fig. 1),

TABLE 3 Template specificity

Template	Concentration (μg per assay)	³ H-dTMP incorporated (pmol)
None	—	—
Activated calf thymus DNA	20	15.2
Native calf thymus DNA	20	3.8
T4 DNA	10	0.8
Hb mRNA	10	—
rRNA	10	—
Yeast tRNA	10	—
poly d(A-T)	4	6.0
poly (dA)·poly (dT)	2	1.1
poly (rA)·poly (dT)	5	—
poly (rA)·oligo (dT)	10	—
poly (dA)	2	0.8
poly (A)·poly (U)	10	0.2
poly (A)	10	0.2

Reaction conditions were as described in Table 1 except for the addition of templates as indicated.

the optimal concentration of MgCl₂ being ten times that of MnCl₂. The effect of monovalent cation concentration is shown in Fig. 2. Although all the monovalent cations tested stimulate the rate of DNA synthesis, KCl gives the greatest stimulation, and NH₄Cl and NaCl are less effective. The optimal concentration for all three monovalent cations is 100 mM at pH 7.0. Both the reticulocyte enzyme and the erythroblast enzymes can use either Mn²⁺ or Mg²⁺ as divalent cation and both are stimulated optimally by 100 mM KCl.

At optimal conditions of pH and ionic strength, the reticulocyte DNA polymerase sediments predominantly at 8S, with a smaller peak of activity at 11S (Fig. 3). This is also the case with the erythroblast enzyme, which has been shown to dissociate from an 11S dimer to an 8S monomer under conditions of salt activation⁷. No DNA polymerase activity sedimenting at 3.4S has been detected. Chang and Bollum have found a high molecular weight DNA polymerase of 6–8S and a low molecular weight DNA polymerase of 3.4S in the cytoplasm of bone marrow and several other tissues, while only the 3.4S DNA polymerase

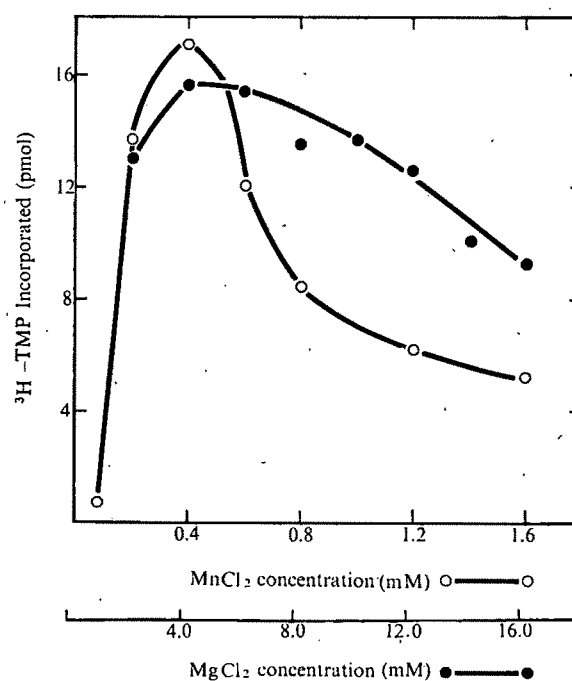


Fig. 1 Effect of divalent cations. Assay conditions were as in Table 1 except for the concentrations of divalent cations. MgCl₂ (●) and MnCl₂ (○).

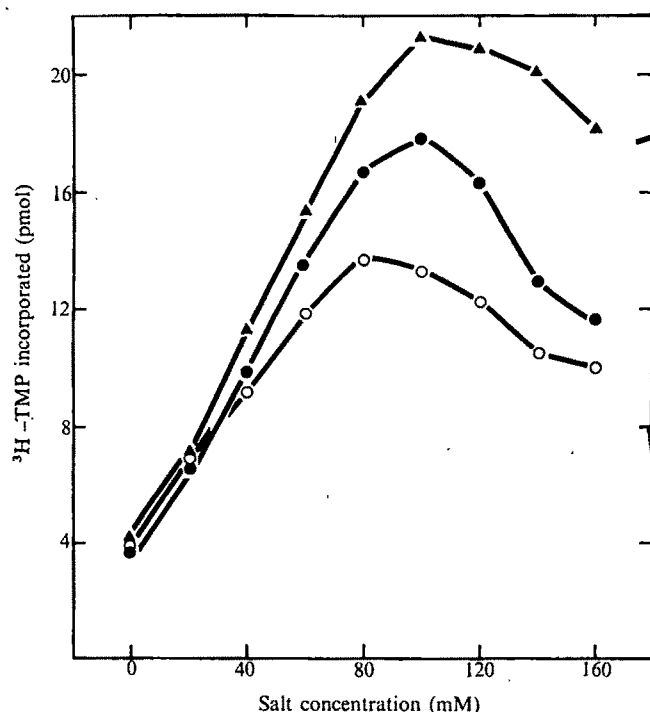


FIG. 2 Effect of monovalent cations. Reaction conditions were as in Table 1 except for the salt concentration. NH₄Cl (●), KCl (▲) and NaCl (○).

was present in the nuclei of these tissues^{5,15}. The absence of a 3.4S DNA polymerase in reticulocytes is consistent with their lack of a nucleus.

The ability of several DNAs, RNAs and synthetic polynucleotides to serve as templates for the reticulocyte DNA polymerase was tested (Table 3). As is the case with the erythroblast cytoplasmic DNA polymerase, activated calf thymus DNA was the best template for the reticulocyte enzymes. However, in contrast to the erythroblast enzyme, poly d(A-T) the alternating copolymer of deoxyadenylate and deoxythymidylate, was less than half as active as activated calf thymus DNA⁷. Double stranded synthetic DNAs such as poly(dA)·poly(dT), or DNA·RNA hybrids were either inactive or very poor templates, as were single stranded RNAs or single stranded homopolymers such as poly(dA) or poly(A). Native calf thymus DNA was less effective as a template than activated calf thymus DNA, suggesting that the reticulocyte enzyme, as well as the erythroblast enzyme, cannot initiate DNA synthesis in the absence of a primer.

Similar to the erythroblast enzyme⁷, the reticulocyte DNA polymerase is markedly inhibited by the rifamycin derivative AF/013 which has also been shown to inhibit the nuclear DNA-dependent RNA polymerases¹⁶⁻¹⁸ as well as the viral RNA-dependent DNA polymerase¹⁸. The synthesis of DNA is also very sensitive to the intercalating dye ethidium bromide, being 50% inhibited at 8 $\mu\text{g ml}^{-1}$. Of particular interest is the inhibitory effect of haem which has been implicated in the control of haemoglobin synthesis in reticulocytes at the translational level^{19,20}. The concentration of haem that completely inhibits the cytoplasmic DNA polymerase (10^{-5} M) is the same as that reported to stimulate globin synthesis optimally²¹.

As with the erythroblast cytoplasmic DNA polymerase⁸, the synthesis of DNA with the reticulocyte enzyme is stimulated by ribonucleoside and deoxyribonucleoside triphosphates. The greatest degree of stimulation is seen with ATP which stimulates the rate of DNA synthesis 3.5-fold, while GTP, UTP and CTP stimulate 2.5 to 3-fold. The α , β - and γ -methylene analogues of ATP are also stimulatory, as is ADP and α , β -methylene ADP. However, 3'-AMP and

5'-AMP have no effect on the reaction. We have previously shown that the erythroblast cytoplasmic DNA polymerase is activated by ribonucleoside diphosphates and triphosphates and that the effect of these activators is to increase the V_{max} of the enzyme rather than to lower the K_m values for the substrates⁸. Furthermore, it has been shown that the mechanism of ATP activation involves the dissociation of the enzyme from an inactive 11.6S dimer to an active 8S monomer. Presumably the reticulocyte enzyme responds to nucleotide activation in the same way. The enzyme isolated from reticulocytes is very similar in its properties to that isolated from the cytoplasm of the nucleated erythroblasts of bone marrow; one difference being that most of the reticulocyte enzyme is not associated with the microsomes even at low ionic strength.

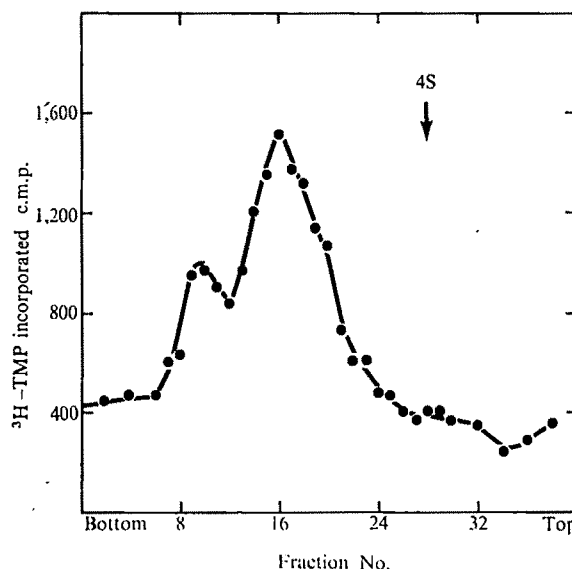


FIG. 3 Sucrose density gradient analysis of reticulocyte DNA polymerase. DNA polymerase was dialysed for 4 h against 5% sucrose buffer containing 10 mM HEPES buffer, pH 7, 1.6 mM MnCl₂, 1.0 mM dithiothreitol and 40 mM KCl. A sample of 0.2 ml of enzyme solution containing 320 μg of protein was layered on a 5–20% sucrose gradient and sedimentation was carried out in a Spinco SW 39 rotor for 15 h at 37,000 r.p.m. at 2°–4° C. Simultaneously 4S tRNA was sedimented as a marker. Thirty-eight equal fractions were collected from the bottom of the tubes and assayed for DNA polymerase activity.

The purification of DNA polymerase from reticulocytes is good evidence that this is in fact a cytoplasmic enzyme. However, the exact role of the cytoplasmic DNA polymerase is not known. It has been proposed that this enzyme is involved in processes of cellular differentiation and gene amplification⁷. This is particularly relevant in an erythroid cell where a large quantity of haemoglobin is synthesised in a relatively short time and no evidence of nuclear gene amplification for this protein has been demonstrated^{22,23}. It is our hypothesis that cytoplasmic DNA and RNA polymerases are involved in the regulation of expression and amplification of genetic information^{7,24}.

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The induction of tumour immunity in mice using glutaraldehyde-treated tumour cells

INDUCING immunity to tumours presents problems similar to those of inducing immunity to infectious microorganisms—the presentation of the agent in an antigenic but non-infectious form. Two approaches have been used to render syngeneic tumour cells non-infectious, lethal irradiation¹ and treatment with chemically reactive groups such as iodoacetamide^{2,3}. These treatments leave the cell metabolising but incapable of division.

Here we demonstrate the use of glutaraldehyde-treated cells for the induction of immunity in syngeneic hosts. Glutaraldehyde solution contains polymeric α and β unsaturated aldehydes resulting from aldol condensation. These react rapidly with protein amino groups in mild conditions and cross link the protein molecules. The modification is irreversible and does not disorder the crystal structure⁴. For these reasons glutaraldehyde has found wide application as a fixative for electron microscopy and X-ray diffraction.

A methyl-cholanthrene-induced BALB/c tumour (Meth A)⁵ was passaged in BALB/c mice by intraperitoneal (i.p.) inoculation. This transplantable tumour is highly infectious in syngeneic animals and a dose as low as 10^8 i.p. will cause ascites and eventual death of the animals. Cells collected from the peritoneum were used for immunisation and challenge. Cells for immunisation were washed in phosphate-buffered saline (PBS), pH 7.0, and made up to 10^8 ml⁻¹. An equal volume of 0.25% glutaraldehyde (Taab Laboratories, Reading, England) in PBS was added. After 10 to 15 min at

22° C, the cells were washed three times by centrifugation. BALB/c female mice were inoculated i.p. with a single injection of 5×10^7 glutaraldehyde-treated cells. Age matched non-immunised mice were kept as controls. After 2 weeks, both groups were challenged i.p. with live tumour cells. Cages containing the different groups were coded before challenge so that the operator could not identify the groups during injection or subsequent inspections.

Non-immunised control mice (Table 1) challenged with 10^8 tumour cells survived for approximately 20 d. Immunised mice were completely protected against this challenge (experiments I, II, III, IV) but not against challenge by 10^6 tumour cells (experiments I and III). In contrast, mice immunised with the same number of irradiated cells showed less protection (experiment IV). In one experiment (II) the glutaraldehyde-treated cells were stored for 2 weeks at 4° C before immunisation. These cells gave similar full protection against the challenge. Mice left for 27 d between immunisation and challenge showed only partial protection (experiment V), indicating that immunity was decreasing at that time.

Preliminary data with a second tumour (a mammary tumour which arose spontaneously in our colony) corroborates these findings. Furthermore, we have found that protection against a higher challenge dose can be achieved with multiple immunisation, whereas mice injected with a similar number of glutaraldehyde-treated syngeneic spleen cells showed no protection. This data will be presented in a subsequent publication.

Two factors might be operating in producing this immunity. First, glutaraldehyde may be preserving the tumour antigenicity and, second, the chemical modification of the proteins may be producing an enhanced cellular immunity at the expense of the humoral response. This phenomenon has been studied in some detail with protein antigens^{6,7}. The implication is that the development of enhancing antibody may be minimal. Further experiments are in progress to study the mechanisms involved.

TABLE 1. The protective effect of glutaraldehyde-treated tumour cells on subsequent challenge with live tumour cells.

Immunisation dose of glutaraldehyde-treated tumour cells	Time before challenge (d)	Challenge dose of live tumour cells (i.p.)	Survivals*			
			Day 0	10	20	30
Experiment I						
5×10^7	14	10^8	5	5	5	5†
Control		10^8	5	5	2	0
Experiment II						
5×10^7	14	10^8	5	5	5	5
(stored cells)†						
Control		10^8	5	5	0	0
Experiment III						
5×10^7	14	10^8	5	5	5	5
		10^6	5	5	0	0
Control		10^8	5	5	1	0
		10^6	5	5	0	0
Experiment IV						
5×10^7	14	10^8	5	5	3	1
(irradiated cells)‡						
5×10^7	14	10^8	5	5	5	5
Control		10^8	5	5	0	0
Experiment V						
5×10^7	27	10^8	4	4	2	2
Control		10^8	5	4	3	0

* Mice developing tumours were usually recognisable before day 10, but only deaths are recorded in this table.

† Surviving mice were observed up to 60 d. No further tumours developed. This group was re-challenged on day 57 with 10^8 tumour cells i.p. None survived.

‡ Treated cells stored for 2 weeks at 4° before immunisation.

§ Cells were washed and resuspended at 5×10^7 ml⁻¹ in PBS immediately before subjecting to 4000 rads γ irradiation from a ⁶⁰Co source.

These preliminary results show that tumour immunity can be induced using glutaraldehyde-treated tumour cells. Apart from the possibly greater immunogenicity, compared with irradiated cells, the treatment is simple and the cells are non-viable. Furthermore, it may be possible to store the treated cells for repeated immunisation. By comparison with previously published data^{2,3,8}, the high degree of protection achieved is sufficiently encouraging to justify experiments with other tumours and other species. If the efficacy can be substantiated the procedure could have advantages in the investigation of tumour immunotherapy in man.

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Fluctuations and distribution of measles virus antigens in chronically infected cells

THE question of chronic measles virus infections in human central nervous system disease has become increasingly important. In subacute sclerosing panencephalitis the role of measles virus has been established¹. In multiple sclerosis the role of measles is less clear although seemingly implicated². It has been suggested that a virus-induced immunopathological process may be involved, initiated by the incorporation of viral antigens into the surface membrane of cells³. The study of measles envelope antigens on the surface of cells in an *in vitro* model is therefore of interest. Here we report the quantitative fluctuations of measles virus antigens at the cell surface in chronically infected cell

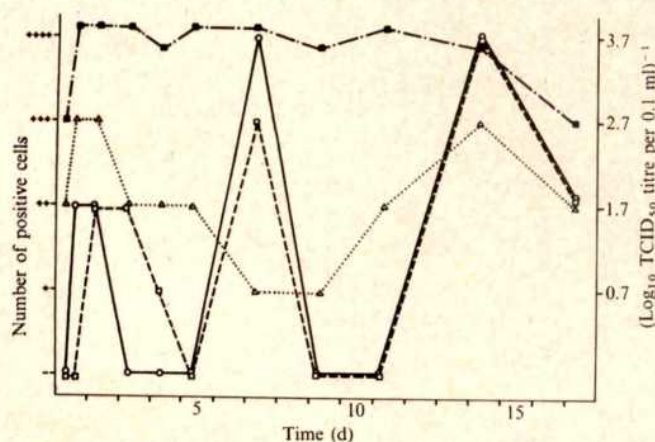


FIG. 1 Fluctuations of membrane immunofluorescence, haemadsorption and extracellular infectivity. ○—○, Immunofluorescence with anti-HL serum; □—□, immunofluorescence with anti-HA serum; ■—■, haemadsorption; △··△, infectivity TCID₅₀ titres.

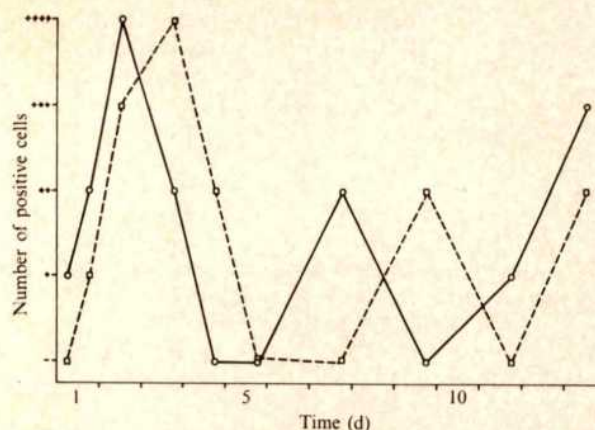


FIG. 2 Fluctuations of surface antigen expression in cells chronically infected with measles virus. Membrane immunofluorescence specific for HL (○) and HA (□).

cultures measured by haemadsorption and indirect membrane immunofluorescence.

A carrier cell line of the Edmonston strain of measles virus in a human heteroploid lung cell line (Lu 106) was used⁴. The cells were maintained for 24 h in Eagle's minimum essential medium (E-MEM) containing 1% L-glutamine, penicillin and streptomycin and 1% calf serum to synchronise cell division before the initiation of individual experiments. Vero cells lytically infected with measles virus were used as controls to test the reliability of the serum, conjugate, and staining technique. Cells were trypsinised in 0.1% trypsin-0.02% versene buffer and suspended in 2% calf serum in E-MEM and 30,000 cells ml⁻¹ were placed on coverslips (10 × 40 mm) within sealed Leighton tubes at 37° C. Uninfected Lu 106 cells were used as controls. The cell cultures approached a monolayer by the end of two weeks. Coverslips were taken for examination every 4 h for the first 12 h and at 24 h intervals for the first 4 d and then on three alternate days each week. A parallel series of extracellular samples were collected for determination of infectivity titres in roller tubes of Vero cells. From serial ten-fold dilutions 0.1 ml samples were taken and inoculated into three tubes per dilution. Final readings were made 14 d after inoculation and the tissue culture infectious dose causing specific cytopathic effects in 50% of the tubes (TCID₅₀) was calculated. Haemadsorption was carried out by washing with phosphate-buffered physiological saline (PBS) without Mg²⁺ and Ca²⁺ and addition of 0.1% African green monkey cells. After adsorption at room temperature for 1 h the coverslips were washed twice with PBS and then examined by light microscopy.

Antisera were prepared by hyperimmunising rabbits (using Freund's complete adjuvant and an intravenous booster after 5 week) with purified whole virus from Vero cells, small particle haemagglutinin (HA) (ref. 5), and virus particles from which surface projections had been removed by treatment with 0.004% trypsin. This treatment removes HA projections but leaves some envelope-associated haemolysin (HL) (Norrby, to be published). Sera were tested by haemagglutination inhibition (HI) and haemolysin inhibition (HLI) tests as described previously⁶. Anti-HA serum had an HI antibody titre of 5,000, but no HLI antibodies demonstrable after removal of HI antibodies by absorption with Tween 80-ether antigen⁶. Anti-HL serum had an HLI titre of 320 and an HI titre of less than 5. Rabbit control serum against uninfected Vero cells was prepared. Before use in immunofluorescence measurements sera were absorbed with non-infected Vero cells.

For membrane immunofluorescence measurements coverslips were washed twice in PBS without Ca²⁺ and Mg²⁺ and incubated with specific antiserum for 30 min at room

temperature. Antiserum was removed by washing twice in PBS; sheep anti-rabbit gamma globulin conjugated to fluorescein isothiocyanate (FITC) (National Bacteriological Laboratory, Stockholm, Sweden) was added for 30 min. After two additional washings in PBS, coverslips were mounted in buffered glycerol on glass slides. The slides were examined immediately with a Zeiss fluorescence microscope. After storage at 4° C a second blind reading was performed by another person at the end of the experiment.

The number of cells showing intense fluorescence was estimated. A grading from + to ++++ was used; + representing 10–25%, ++ 25–50%, +++ 50–75%, ++++ more than 75%. Periodic fluctuations in the numbers of cells displaying membrane HA occurred in all four experiments (Figs 1 and 2). During the first 2–4 d the number of positive cells increased to a maximum of ++ to ++++. Thereafter fluorescence disappeared and remained absent for 1–2 d. Fluorescence then reappeared independently of growth medium changes. During the 2 week observation period one more cycle of disappearance and reappearance was encountered in three experiments.

Membrane fluorescence specific for HL antigen fluctuated in two experiments (Figs 1 and 2). It remained absent throughout the other two experiments despite variations of the HA antigen. HL antigen expression was occasionally found in the absence of HA antigen (Fig. 2).

The titre of extracellular infectious virus varied but did not show a distinct correlation with presence or absence of membrane antigens as measured by immunofluorescence (Fig. 1). Intracellular antigen and haemadsorption fluctuated only a little and never occurred in fewer than 75% of cells. Haemadsorption therefore was a more sensitive indicator of surface measles antigen than membrane immunofluorescence.

A polar distribution of antigen (capping) commonly occurred in single cells or in cells appearing to undergo division (Fig. 3a and b). In Fig. 3b there is polar fluorescence at opposite ends of what may be a cell in mitosis. In other cells the discontinuous membrane immunofluorescence was most common (Fig. 4a and b). The relatively smaller syncytium in *a* displays a more intense membrane immunofluorescence than the syncytium in *b*. This was generally true.

Several membrane antigens have the capacity to redistribute^{7–9}. Marcus found viral protein at one end of cells by haemadsorption in NDV-infected cells¹⁰. Capping of viral antigen might be of some interest with reference to a possible occurrence of antigenic modulation⁹.

Various factors such as cell growth^{11,12}, phases of the cell cycle^{11,12}, energy generating system¹³ and pinocytosis of antigen-antibody complexes after capping⁹, have been

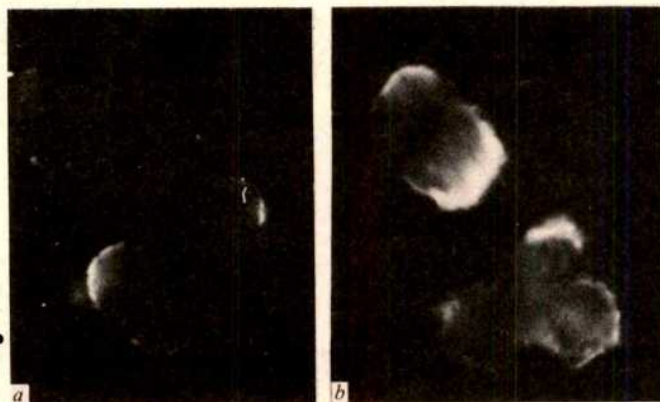


FIG. 3 *a* and *b*. Capping of measles virus HA antigen demonstrated by indirect membrane immunofluorescence. Magnification $\times 930$.

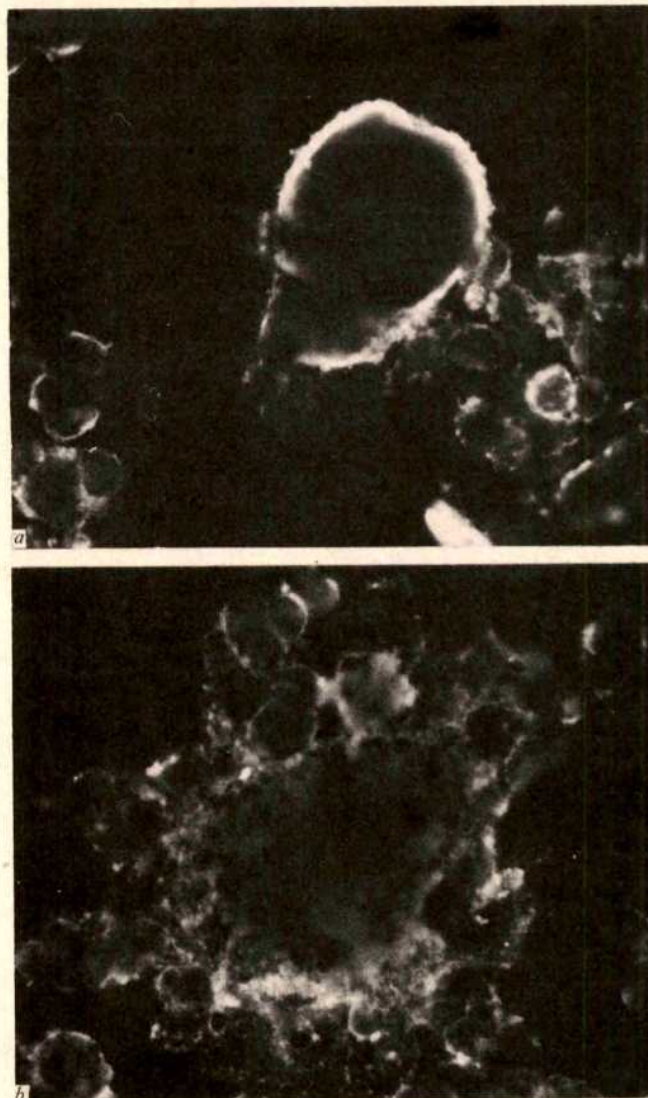


FIG. 4 Membrane antigens on single cells and syncytia in a culture chronically infected with measles virus, demonstrated by the indirect immunofluorescence technique using an anti-'whole' virus serum. Magnification $\times 750$.

found to cause fluctuating expression of different surface antigens. In a cell line chronically infected with virus, factors such as autointerference or interferon production^{14,15} may also be of importance. It has been observed (A. E., to be published) that carrier Lu 106 cells display a varying sensitivity to measles virus-specific cytotoxic antibodies. This may relate to the variable expression of membrane antigen as shown by immunofluorescence.

In multiple sclerosis the pathogenesis and aetiology of the disease is not known, but it is possible that it represents a chronic infection and that the exacerbations and remissions characteristic of the disease are the result of irregular expression of virus-specific antigens in myelin membranes and an immunological attack on these antigens.

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Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans

CHANGES in brain tryptophan (Trp) associated with feeding are relevant to serotonin (5-hydroxytryptamine, 5-HT)-mediated function, because in the brain Trp levels modulate 5-HT synthesis¹. Using rodents, some investigators²⁻⁵ have confirmed our observations^{6,7} that after ingestion of balanced diets plasma Trp increases but brain Trp decreases or is unchanged. Other such dissociations between plasma and brain Trp have been observed during fasting, and after eating in hypophysectomised rats, indicating that the changes are not secondary to stress or pituitary mechanisms⁷. The dissociation is also independent of diurnal or nocturnal variations in plasma Trp^{4,7}, but there is no explanation for it.

On the other hand, ingestion of pure carbohydrate (CHO) diets⁸⁻¹⁰, and injection of Trp⁵ and drugs that affect 5-HT metabolism¹¹ increase both total plasma and brain Trp. This

led to the hypothesis that levels of total plasma Trp reflect levels of brain Trp⁸. The hypothesis has been challenged by evidence that injections of Trp increase free plasma much more than total plasma Trp⁵.

A further challenge is the report that CHO diets increase total plasma and brain Trp, but decrease free plasma Trp¹⁰. Some drugs that increase brain Trp and 5-HT metabolism do not increase free plasma Trp¹². Furthermore, transport of Trp from plasma to brain depends on levels of competing plasma amino acids⁹ and on the ratio between total plasma Trp and neutral amino acids (NAA) in plasma⁹. So far, there is general agreement that after ingestion of balanced diets^{2,3,5} or CHO diets^{10,13}, free plasma Trp is decreased, and total and bound plasma Trp are increased.

Tryptophan is an essential amino acid, normally binding to albumin in plasma¹⁴. Displacement of Trp from the binding site in the albumin molecule by free fatty acids (NEFA)^{2,3,10}, and by drugs that decrease plasma Trp but increase brain Trp^{11,15}, is a suggested cause of plasma-brain Trp dissociations. It has been speculated that CHO and insulin increase the albumin-binding of Trp by decreasing the extent of saturation of albumin with NEFA¹⁰. Decreases of plasma NEFA after ingestion of balanced diets or insulin and CHO diets are well known¹⁶. Mobilisation of NEFA can also be influenced by concentrations of plasma triglycerides of hepatic or dietary origin¹⁶. A role of NEFA in controlling food intake has been postulated¹⁷, but whether plasma Trp (free or bound) is concerned with the control of food intake remains to be seen. In humans, injection of insulin increases total plasma Trp and decreases plasma NAA¹⁸, and CHO ingestion reduces plasma NEFA and free plasma Trp¹³.

We have investigated the mechanisms mediating plasma-brain Trp dissociations and looked for similar relationships between total, bound and free plasma Trp and cerebrospinal fluid (CSF) in human subjects after intake of a balanced diet.

Twenty-nine subjects with Parkinson's disease, Huntington's chorea or related neurological disorders volunteered for diagnostic studies of monoamine metabolism. They were fasting and recumbent in bed for 12 h before CSF was drawn by lumbar puncture (at 1000 h), and samples were mixed with 2% ascorbic acid and cooled to -20°C. Blood was drawn by venipuncture and centrifuged. The plasma was stored at -20°C. This procedure was repeated at 1600 h. Between sampling all subjects remained in bed; nine fasted all the

TABLE 1 Total, bound and free plasma Trp, CSF-Trp and CSF-5-HIAA in man during fasting and after feeding

Conditions	Total μg ml ⁻¹ ± s.e.m.	Plasma Trp Bound μg ml ⁻¹ ± s.e.m.	Free μg ml ⁻¹ ± s.e.m.	CSF-Trp ng ml ⁻¹ ± s.e.m.	CSF-5-HIAA ng ml ⁻¹ ± s.e.m.
Diet a					
Fasting 12 h (1000 h)	10.95 ± 0.87(14)	9.38 ± 1.14(7)	0.55 ± 0.09(7)	377.30 ± 41.40(14)	49.20 ± 4.80(14)
Postprandial (1600 h)	14.44 ± 1.01(14)	12.36 ± 0.38(7)	0.20 ± 0.06(7)	303.90 ± 31.00(14)	39.60 ± 3.30(14)
% Change	32%****	32%****	-64%****	-20%****	-20%***
Diet b					
Fasting 12 h (1000 h)	12.08 ± 0.38(6)	8.92 ± 0.19(6)	0.49 ± 0.02(6)	269.00 ± 10.44(6)	27.18 ± 3.79(6)
Postprandial (1600 h)	17.25 ± 1.54(6)	14.99 ± 1.52(6)	0.35 ± 0.02(6)	211.66 ± 17.41(6)	17.63 ± 3.68(6)
% Change	43%****	68%****	-29%***	-21%*	-35%*

Total plasma Trp was isolated¹⁹ and bound and free (ultrafiltrate) plasma Trp were separated as before²⁰. CSF-Trp and the main metabolite of 5-HT in CSF, 5-hydroxyindoleacetic acid (5-HIAA), were separated in a Dowex 50, Na⁺ resin²¹.

Plasma NAA (valine, isoleucine, leucine, tyrosine and phenylalanine) were separated by column chromatography with a Beckman model 120 C, amino acid analyzer. The plasma ratios between Trp (total, bound or free) and NAA were obtained by dividing Trp in plasma by the sum of the five plasma NAA⁹. Trp and 5-HIAA were determined fluorometrically as described elsewhere^{7,11}. Total serum protein, albumin, and triglycerides were determined by the clinical laboratory of NIH. Statistical analyses were done using paired Student's *t* tests and linear correlations²².

Numbers in parenthesis are number of subjects.

s.e.m., standard error of the mean²².

Statistical significance, * *P* < 0.05, ** *P* < 0.02, *** *P* < 0.01, **** *P* < 0.001.

% Change, represents the percentage change between fasting and postprandial levels.

TABLE 2 Plasma levels of neutral amino acids, Trp, and ratios of total and free plasma Trp to neutral amino acids, before and after feeding

(A)		
Plasma amino acids (NAA)	12 h fasting (1000 h) $\mu\text{g ml}^{-1} \pm \text{s.e.m.}$	Postprandial (1600 h) $\mu\text{g ml}^{-1} \pm \text{s.e.m.}$
Valine (V)	21.7 ± 2.0	28.8 ± 2.0 (32.7%)*
Isoleucine (I)	7.3 ± 0.9	11.8 ± 1.1 (61.6%)**
Leucine (L)	11.2 ± 1.0	17.9 ± 1.4 (59.8%)**
Tyrosine (T)	7.9 ± 0.6	11.4 ± 0.6 (44.3%)**
Phenylalanine (P)	8.9 ± 0.5	11.6 ± 0.6 (30.0%***)
Tryptophan (Trp)	12.1 ± 0.4	17.3 ± 1.5 (42.5%***)
(B)		
Ratio	Fasting (1000 h)	Postprandial (1600 h)
Total plasma Trp		
Sum of NAA	0.2156 ± 0.0108	0.2189 ± 0.0139 (1%) NS
Free plasma Trp		
Sum of NAA	0.0086 ± 0.0012	0.0043 ± 0.0002 (-50%)**

Each value represents the average of six subjects \pm s.e.m.

%, Percentage change between fasting and postprandial samples.

Sum of NAA equals sum of V + I + L + T + P in $\mu\text{g ml}^{-1}$.

Statistical significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

time; at 1200 h fourteen ate a random balanced diet, hospital lunch (diet *a*, 25 g protein, 25 g fats, 55 g carbohydrates, totalling 545 calories); six received equivalent calories in the form of 60 g roast turkey, 100 g steamed rice, 20 g bread, 10 g butter, 100 g unsweetened apple sauce and 240 ml of whole milk (diet *b*) to control for the randomness of the diet. As Table 1 shows all subjects who received diet *a* and diet *b* showed statistically significant changes between the fasting (1000 h) and postprandial (1600 h) samples: postprandial levels of total and bound plasma Trp increased whereas free plasma Trp levels were consistently decreased. Postprandial levels of CSF-Trp and CSF-5-HIAA were also decreased in the 1600 h samples. Significant negative correlations between total or bound plasma Trp and CSF-Trp or CSF-5-HIAA were obtained regardless of diet and it is unlikely that these changes are due to a diet artefact. Positive non-significant correlations between free plasma Trp and CSF-Trp or CSF-5-HIAA were obtained. If Table 1 is interpreted without considering the food intake status of the subjects, a negative correlation between total plasma Trp and CSF-Trp or CSF-5-HIAA is still obtained during the two samplings, but the correlations between free plasma Trp and CSF-Trp and CSF-5-HIAA were only positive during the fasting (1000 h) samplings. The percentage changes during the postprandial period in total and bound plasma Trp and CSF-5-HIAA were greater with diet *b*, whereas they were greater for free plasma Trp with diet *a*. In nine subjects who fasted throughout the test, no significant postprandial changes in CSF-5-HIAA were observed between the two samples (difference: 0.70 ± 3.5 ng/ml $^{-1}$, $P > 0.05$). CSF-Trp or total plasma Trp was not measured in these subjects, but it is also unlikely that the postprandial changes in total, bound, and free plasma Trp and CSF-Trp are due to diurnal variations^{4,7}.

In subjects eating diet *b*, plasma levels of valine, isoleucine, leucine, tyrosine and phenylalanine increased significantly as illustrated (Table 2A). The greatest postprandial increments in plasma NAA were observed in isoleucine and leucine (61.6% and 59.8%, respectively), and the smallest in valine and phenylalanine fractions (32.7% and 30.0%, respectively). As Table 2B shows, the postprandial ratio of total plasma Trp to NAA was not changed, whereas the postprandial ratio of free plasma Trp to NAA was reduced (-49.5%, $P < 0.01$). There was significant positive correlation between the ratio of free Trp to NAA and CSF-Trp or CSF-5-HIAA, but not between the ratio of total plasma Trp to NAA and CSF values.

Since Trp binds avidly to plasma albumin, an increase in this protein can influence the transport of this amino acid into brain. All subjects showed an increase in serum proteins and plasma albumin after feeding. Serum proteins were in-

creased from 6.70 ± 0.37 g per 100 ml to $7.05 \pm .40$ g per 100 ml. Serum albumin was increased from 3.86 ± 0.018 g per 100 ml to 4.04 ± 0.17 g per 100 ml. These increments in plasma albumin represent an increase in the availability of binding sites for plasma Trp. Although NEFA were not measured, it is well known that ingestion of balanced or CHO diets decreases plasma NEFA¹⁶. Measurements of the plasma triglycerides were considered more valuable, because diets rich in CHO produce significant increments in plasma triglycerides which influence the mobilisation of NEFA from fat and liver²³. All subjects developed a significant postprandial hypertriglyceridaemia. Serum triglycerides increased from 87.66 ± 10.80 mg per 100 ml to 136.00 ± 18.25 mg per 100 ml. The increment in dietary and liver triglycerides after feeding is probably responsible for the significant decrease in plasma NEFA, and hence may influence the albumin-binding of Trp by decreasing the extent of saturation of albumin by NEFA.

Determinations of precursors such as Trp, and metabolites of 5-HT such as 5-HIAA, in lumbar CSF have been used widely to estimate the metabolic state of 5-HT in the human central nervous system, where more direct measurements are impossible²⁴. There is abundant evidence that changes in lumbar CSF reflect changes in brain²⁵. It is now certain that lumbar CSF-5-HIAA does not all derive from the lumbar cord as reported by Bulat *et al.*²⁶, and using their experimental conditions it has been shown that 40% to 50% derives from brain 5-HIAA²⁷. It has also been estimated that in humans, 23% to 37% of the lumbar CSF-5-HIAA is derived from spinal 5-HIAA²⁸. The decrease of CSF-Trp and CSF-5-HIAA after feeding suggests that similar changes occur in the human brain. These facts suggest that the synthesis of brain 5-HT is decreased after ingestion of food in humans. In rodents balanced diets produce a decrease in the synthesis of 5-HT⁷. Our values of CSF-Trp and CSF-5-HIAA are within the range of values reported by others for human CSF²⁴.

There is general agreement that CHO or balanced diets increase plasma Trp (total and bound) and decrease free Trp and NEFA in the plasma^{8,10}. It is obvious that an increment in NAA is associated with a decrement in brain Trp, and that perhaps the ratios of total plasma Trp to NAA or free plasma Trp to NAA more accurately reflect the changes in brain Trp. In CHO diets we have found that although the levels of free plasma Trp were decreased, the ratio of free plasma Trp to NAA and the ratio of total plasma Trp to NAA were significantly increased ($P < 0.01$, manuscript in preparation). In humans, the ratio of total plasma Trp to NAA did not reflect changes in CSF-Trp or CSF-5-HIAA, whereas the ratio of free plasma Trp to NAA

was significantly correlated with changes in CSF values. This indicates that the ratio of free plasma Trp to NAA is the most important factor controlling the transport of plasma Trp into brain, and that this ratio reflects changes in brain Trp not reflected by either total plasma or free plasma Trp. This hypothesis does explain why CHO diets should increase brain Trp while routine diets should decrease it. In CHO diets free Trp to NAA is increased and in balanced diets the free Trp to NAA is decreased. Further experimentation will be necessary to establish definitively the generality of this new hypothesis.

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Localisation of phenylethanolamine N-methyl transferase in the rat brain nuclei

THE conversion of noradrenaline to adrenaline is catalysed by phenylethanolamine N-methyl transferase (PNMT, EC 2.1.1)^{1,2}, which is highly localised in the adrenal medulla. Small amounts of PNMT-like enzyme³⁻⁵ and adrenaline^{6,7} have been detected in the central nervous system, and we

have now been able to study the distribution of the enzyme in specific nuclei of the rat brain. This has been made possible by the development of a technique for the dissection of small areas and individual nuclei⁸ of the brain, and the availability of a sensitive assay for the measurement of PNMT. Our findings suggest that adrenaline may serve as a neurotransmitter in the central nervous system.

Sprague-Dawley male rats (200–250 g) were killed by decapitation, and their brains were removed immediately and frozen on dry ice. Frontal serial sections 300 μ m thick were cut from the border between the spinal cord and the medulla oblongata, at the level of the pyramidal decussation, as far rostral as the level of the anterior commissure. Regions rich in catecholamine-containing cell bodies were removed under stereomicroscopic control⁸ as follows. The A1 region⁹ (equivalent to the C1 region of Hökfelt *et al.*¹⁰), was located in the lateral reticular nucleus of the medulla oblongata, between the pyramidal tract and the tractus spinalis nucleus trigemini just under the ventral surface of the medulla oblongata. (Details of the various dissections mentioned here will be published elsewhere.) The A2 region (probably the same as the C2 region of Hökfelt *et al.*¹⁰) contains catecholamine cell bodies at the level of the region of the vagal nuclei (nucleus commissuralis, nucleus originis dorsalis vagi, nucleus tractus solitarii), and was removed from an area just dorsolateral to the central canal. The A5 region contains catecholaminergic cell bodies, most of which lie between the superior olive and the facial nerve, close to the ventral surface of the brain. The A6 region (locus coeruleus), was removed from the tegmentum medial to the superior cerebellar peduncle. The A7–A8 cell bodies (part of the nucleus cuneiformis) are found at the level of the border of the mesencephalon and pons, dorsal to the medial lemniscus and medial to the lateral lemniscus.

Cortical, limbic and hypothalamic regions were removed with a microdissecting knife. Samples from the septum, pre-optic area and caudate-putamen were removed from sections at the anterior commissure level, where the septum is delimited by the lateral ventricles, the corpus callosum and the anterior commissure. The preoptic region lies between the anterior commissure and the ventral surface of the brain medial to imaginary vertical lines drawn through the lateral ventricles.

Samples of tissue from the medial basal hypothalamus (including median eminence, arcuate nucleus and a part of the ventromedial nuclei) were dissected from sections at the median eminence level. The amygdala was removed from the same section, and was limited by the optic tract medially, the caudate-putamen dorsally and the external capsule-piriform cortex laterally. Samples were dissected from the cortex and hippocampus.

The hypothalamic nuclei, the olfactory tubercle and the nucleus interstitialis striae terminalis, were removed, and both the compact and reticular zones of the substantia nigra were dissected between the lemniscus medialis and the crux cerebris.

PNMT in brain samples was measured by a modification of the method previously described² as follows. Brain tissue from one rat was homogenised in 65 μ l of 0.2 M Tris-HCl buffer, pH 8.6, and 5 μ l was removed for protein determination¹¹. After centrifugation, 50 μ l of the supernatant was transferred to 13-ml glass-stoppered centrifuge tubes. The reaction was initiated by addition of 100 μ l of a solution containing 4.5 μ mol of phenylethanolamine and 0.54 nmol of ³H-methyl-S-adenosyl-1-methionine (New England Nuclear Corp., specific activity 4.5 mCi μ mol⁻¹). Blanks were prepared by omitting the substrate from the incubation mixture. The tubes were incubated for 30 min at 37° C. The reaction was stopped by the addition of 0.5 M borate buffer, pH 10, and the radioactive product was separated from the ³H-methyl-S-adenosyl-1-methionine by extraction into toluene

TABLE 1 PNMT activity in rat brain nuclei

Region	PNMT
A1 (C1)	41.5 \pm 5
A2 (C2)	40.3 \pm 4.3
A5	5.6 \pm 1.4
A6 (locus coeruleus)	12.0 \pm 1.2
A7-A8	6.1 \pm 1.1
Cerebellar cortex	Not detectable
Frontal cortex	Not detectable
Cingulate cortex	Not detectable
Piriform cortex	Not detectable
Hippocampus	Not detectable
Amygdala	Not detectable
Septum	6.3 \pm 1.7
Nucleus interstitialis striae terminalis	6.7 \pm 0.8
Olfactory tubercle	Not detectable
Preoptic region	7.7 \pm 0.7
Hypothalamus	
Basal hypothalamus	15.9 \pm 2.2
Nucleus periventricularis	8.0 \pm 0.9
Medial forebrain bundle (anterior)	6.2 \pm 0.5
Nucleus dorsomedialis	10.9 \pm 3.2
Nucleus suprachiasmatis	5.6 \pm 0.7
Nucleus supraopticus	5.2 \pm 1.3
Nucleus anterior	5.9 \pm 1.3
Nucleus paraventricularis	15.0 \pm 2.5
Area retrochiasmatica	9.7 \pm 3.4
Median eminence	15.6 \pm 3.0
Nucleus arcuatus	10.3 \pm 2.0
Nucleus ventromedialis	8.3 \pm 1.6
Nucleus perifornicalis	8.1 \pm 1.6
Nucleus premammillaris dorsalis	4.1 \pm 0.5
Nucleus premammillaris ventralis	4.9 \pm 1.4
Nucleus posterior hypothalami	5.1 \pm 1.0
Medial forebrain bundle (posterior hypothalamic level)	5.3 \pm 1.2

Brain samples were dissected and processed as described in the text. Results represent mean \pm s.e.m. for groups of eight animals. One unit of enzyme activity is equal to 1 pmol of ³H-N-methyl phenylethanolamine formed per mg of protein and per hour of incubation.

containing 3% of isoamyl alcohol (v/v). A 5 ml sample of the organic solvent was evaporated to dryness under a stream of air, and the radioactivity remaining was counted. The whole procedure was repeated using internal standards consisting of 1 U of purified PNMT¹³, and the results were corrected accordingly. The use of a methyl donor of high specific activity and a drying procedure to eliminate a volatile radioactive contaminant increased the sensitivity of the assay fifty-fold over that previously in use¹³.

No enzymatic activity was found when phenylethanolamine was replaced by β -phenylethylamine or tryptamine as substrates for the reaction. This finding suggests that the enzyme studied is PNMT, rather than the non-specific N-methyl transferase described earlier¹².

Our results indicate that PNMT activity is unevenly distributed in the rat brain, and is localised in specific areas. The greatest PNMT activity was measured in two areas, A1 and A2, which are rich in catecholamine-containing cell bodies⁹. Other areas containing many catecholaminergic neurones, such as A5 and A7-A8, were low in PNMT activity, and the region of the locus coeruleus (A6) showed intermediate values (Table 1). These results indicate that PNMT is not distributed homogeneously among regions rich in catecholamine-containing neurones.

PNMT activity could not be detected in any of the cortical areas examined, in the caudate, the amygdala or the olfactory tubercle (Table 1). The substantia nigra, the nucleus interstitialis striae terminalis and the septum showed relatively low levels of activity.

Enzymatic activity was detected in all of the hypothalamic nuclei studied as well as in the preoptic region. The PNMT activity was found to be lower than the dopamine- β -hydroxy-

lase and tyrosine hydroxylase activities found in similar hypothalamic nuclei.

PNMT was found to be unevenly distributed within hypothalamic nuclei. The largest concentrations were in the medial basal hypothalamus in the arcuate and paraventricular nuclei and in the median eminence. Intermediate concentrations were found in the paraventricular, dorsomedial, ventromedial and perifornical nuclei and in the area retrochiasmatica. Small concentrations were found in the rest of the hypothalamic nuclei.

Adrenaline and PNMT are present in high concentrations only in the adrenal medulla^{1,2}. PNMT and its product seem also to be present in nerves, however. Work with amphibians has provided evidence that PNMT is synthesised and transported in the axons of peripheral nerves¹⁴. Small quantities of PNMT²⁻⁵ and adrenaline^{6,7} have been measured in the brain of mammals. Hökfelt *et al.*¹⁰ reported an immunohistological technique for the visualisation of PNMT in the brain. The localisation of the enzyme that we report agrees well with the histological data.

The medial basal hypothalamus, a region rich in the catecholamines dopamine and noradrenaline (M.P. *et al.*, submitted for publication), plays an important role in neuroendocrine regulation. The fact that PNMT is concentrated in this region suggests that adrenaline can also be produced there. Thus adrenaline may have a role in the hypothalamic control of the anterior pituitary.

J.M.S. is a visiting scientist at the National Institute of Mental Health, and M.J.B. is a research associate in the Pharmacology-Toxicology Program of the National Institute of General Medical Sciences.

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Learning impairment in rats after 6-hydroxydopamine-induced depletion of brain catecholamines

STEIN¹ suggested, on the basis of the effects of amphetamine and other adrenergic drugs on self-stimulation behaviour, that the reinforcement feedback of the consequence of an action was mediated by a noradrenergic pathway in the region of the medial forebrain bundle. Noradrenergic and dopaminergic pathways in this region of the brain have been mapped by Ungerstedt², and Crow^{3,4} has suggested that the gustatory and olfactory input to these pathways might fit them for a role in transmitting environmental feedback. Other evidence implicates the dopaminergic nigro-striatal pathway in memory function as well. Microstimulation in the pars compacta of the substantia nigra has been found to produce a memory impairment not found on stimulation of the surrounding nuclei⁵, lesions in the substantia nigra impair learning^{6,7} and pharmacological manipulation of the level of dopamine in the substantia nigra can impair learning and performance of rats in a delayed response task⁸. Crow⁹ has found that locus coeruleus lesions may completely abolish learning in a runway situation. This indicates the involvement of monoaminergic-containing neurones in memory function. 6-Hydroxydopamine produces a depletion of brain noradrenaline and dopamine when injected into the lateral ventricle of the rat brain¹⁰, and also a reduction in the activity of the biosynthetic enzyme tyrosine hydroxylase¹¹ consistent with the morphological finding of acute degeneration of the nerve terminals^{12,13}. Depletion of the monoaminergic neurones using 6-hydroxydopamine would therefore be predicted to impair learning behaviour, and has indeed been found to produce a transitory decrease in responding on a fixed-ratio schedule for water reward¹⁴. This, however, returned to normal within a few days. Conversely, an increase in responding on a variable interval schedule after 6-hydroxydopamine has been found¹⁵.

This latter effect, however, was shown to be on the emission of responses after training to a stable level and not on the learning process itself. Our results using a demanding motor-learning task, show severe impairment after 6-hydroxydopamine during acquisition itself, which is not due to gross motor paralysis or hypokinesia.

Twenty experimentally naive male albino Wistar rats, weighing about 200 g at the time of operation, were anaesthetised with ether and two doses of 6-hydroxydopamine, (150 µg dissolved in 15 µl of 0.9% saline with 1 mg ml⁻¹ ascorbic acid antioxidant) were injected, one each side, into the lateral ventricle 2 mm lateral and 1–2 mm posterior to the bregma in ten animals. Controls received injections of the vehicle solution. Both groups were pretreated with the monoamine oxidase inhibitor tranylepromine (5 mg kg⁻¹) 30 min before the operation to enhance the depletion of dopamine¹⁶.

Two treated animals died during the course of the experiment. Pretraining was begun approximately 10 weeks after treatment, acquisition started 2 weeks later and transfer 4 weeks after this. The animals were trained on a complex task in which they had either to pull or to push a ball out of a perspex tunnel to reach food¹⁷. The apparatus comprised two open-topped boxes 25 cm high, and base 22 cm × 25 cm, connected at floor level by a 23 cm long cylindrical plexiglass alley of inside diameter 10 cm. The boxes were identical except that one, the goal box, contained a food dish into which sugar pellets could be delivered by a Gerbrands feeder. The alley could be partially obstructed by a plastic ball, 8 cm in diameter and weighing 45 g, placed equidistant from the start and goal boxes. Attached to the ball by a rubber suction cup was a 3.8 cm handle made from a 4 BA screw. Movement of the ball in either direction could be blocked by metal pegs mounted on pivots over the top of the alley and protruding down from the roof through a longitudinal slit in the plexiglass. Both pegs were always left hanging down, although only one was locked so that the rat had no obvious clue as to which of the two responses was possible on any trial. The apparatus was weakly illuminated and the rat was observed by mirrors placed at 45° above the apparatus. The subjects were placed on a 2 h d⁻¹ food access schedule until their weights were approximately 85% of free feeding weight. Subsequently they were fed 15 g d⁻¹ of laboratory chow, immediately after the subject's last trial of the day. On day 1 of pretraining, the subjects were placed in the start box and given 5 min exposure to the apparatus with the ball removed, pegs hanging down and food cup overflowing with sugar pellets. Fifteen further trials were given in which now only 2 pellets were available in the food cup per trial. A further 34 trials were given in which the pellets were delivered by the Gerbrands feeder, one on entry to the goal box and another 4 s later. Acquisition testing was now started with the ball placed in the tunnel and the distal peg locked so that the ball could only be pulled out of the tunnel. The subject was given a 5 min trial to solve the problem and then removed from the apparatus if the ball was still in place. If the ball had been moved part of the way out by the end of 5 min a further 5 min was allowed, but the subject unconditionally removed at the end of this time. The amount of time spent actually in contact with the ball was noted, this being taken as a measure of how much the rat was trying to move the ball ('trying time') and hence being a better measure than 'total-time-in-the-apparatus-to-solution' which is made up of behaviour other than that oriented towards the ball. The movements of head and paws used in manipulating the ball were also noted. Forty-seven trials were given in all, and the cumulative 'total-time-spent-in-the-apparatus-before-solution' of the task noted for each animal. By trial 28, one treated and two controls had failed to solve the task and showed no sign of doing so. 'Shaping trials' in which the ball was placed half in and half out of the tunnel and then moved further back were given. These animals were classified as

TABLE 1 Performance on ball-in-tunnel task.

	Acquisition		Transfer one		Transfer two	
	Time to solution (min)	Trying time (s)	Time to solution (min)	Trying time (s)	Time to solution (min)	Trying time (s)
6-hydroxydopamine (n = 8)	65.8 ± 14.7	572 ± 126	45 ± 15	29 ± 12	6.8 ± 2.4	4.3 ± 0.9
Controls (n = 8)	8.75 ± 1.9 P, 0.05	80 ± 14 P, 0.001	87 ± 26 Not significant	49 ± 18 Not significant	7.0 ± 0.5 Not significant	4.9 ± 0.5 Not significant

Performance of controls and 6-hydroxydopamine treated animals on acquisition, transfer 1 and transfer 2 of the ball-in-tunnel task. 'Time to solution' is the total time spent in the apparatus before solving the task; 'trying time' is the time spent in contact with the ball manipulating it. Values given are means and standard deviations for each group.

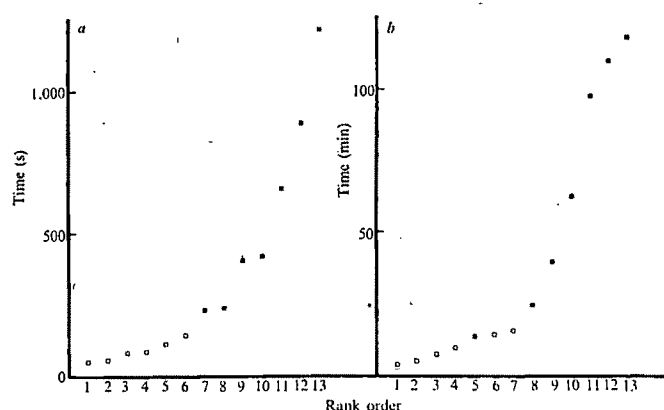


FIG. 1 Trying time (a) and time in apparatus (b) for the control and treated animals plotted against their position in the overall rank order of times for both groups. The animals separate out into two distinct groups, that is rapid solution controls and slow solution treated, with very little overlap. Of the 16 subjects used, two controls and one treated failed to solve acquisition within 28 trials and were dropped from the analysis of acquisition times. □, Controls; ■, treated.

failures and dropped from the analysis of acquisition. At the end of 47 acquisition trials, the proximal peg was locked and the other released and transfer testing begun with the animals being transferred from pull to push during the course of 28 trials. A second transfer phase was then started in which the animals were transferred back to pull. The total time in the apparatus for successful transfer was noted in each case. (Table 1).

On both 'trying time' and 'total-time-in-apparatus-to-solution' the 6-hydroxydopamine animals were severely impaired relative to the controls. (Fig. 1). On both transfer 1 and transfer 2, there was no significant difference between the groups, all animals mastering the second transfer within one 5 min trial. At the end of transfer 2 the animals were killed by decapitation and the brains rapidly removed, chilled and dissected into regions according to the method of Glowinski and Iversen¹⁸. The striatum, hypothalamus and cortex were homogenised in distilled water (1:10(w/v)) and 10 µl aliquots taken for radiochemical assay of tyrosine hydroxylase^{19,20}, the results of which confirmed that the 6-hydroxydopamine animals had sustained severe losses of adrenergic neurones in the hypothalamus, cerebral cortex and corpus striatum (Table 2).

The severe impairment in acquisition in the treated animals cannot be attributed to a motor impairment. The general mobility and time spent in the tunnel was the same in both groups. Each treated animal showed the normal range of head and paw movements and the frequency of these movements was the same in both groups. It also seems unlikely that the deficit is motivational as the treated animals spent as much time as controls in behaviour orientated towards the ball (measured as cumulative time in physical contact with the ball for each trial). Although this measure decreased after the first few trials as the animals habituated to the ball, it decreased equally for both groups, from an initial value that was virtually identical for both treated and control groups.

TABLE 2 Tyrosine hydroxylase activity (DOPA formed pmole h⁻¹ g⁻¹ wet weight).

	Striatum	Hypothalamus	Cortex
6-Hydroxydopamine (n = 8)	35.6 ± 12.0 (8.1%)	19.4 ± 4.5 (16.4%)	2.48 ± 2.32 (14.9%)
CONTROLS (n = 8)	438 ± 25.5	118 ± 8.7	16.7 ± 2.4

Mean and standard error of the tyrosine hydroxylase activity of the three brain areas (see text). These values in the 6-hydroxydopamine-treated animals are also expressed as % control value for that region.

Nor do the treated animals suffer from a retention defect, as once they had mastered the task they, without exception, performed perfectly on every subsequent trial and did not show the pattern of occasional failures expected with a retention defect. Thus 6-hydroxydopamine treatment produced a severe impairment in the actual learning process itself, as distinct from retention or emission of responses. Two related questions remain for future research. First, is the deficit specific to learning the complex motor sequences required for this demanding task, or general to all learning dependent on reinforcement? Second, dopaminergic systems are implicated in complex motor functions and noradrenergic systems with reinforcement mechanisms. The intraventricular 6-hydroxydopamine treatment severely depletes both dopamine and noradrenaline from the forebrain and selective lesion techniques using 6-hydroxydopamine will be employed to determine whether one amine rather than the other underlies the learning deficit.

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Activation of human T and B lymphocytes by polyclonal mitogens

LYMPHOCYTES can be activated *in vitro* by a variety of so-called polyclonal stimulants or mitogens (reviewed in refs 1, 2 and 3). These responses characteristically involve a larger proportion of cells than observed with antigen as the eliciting ligand, and the initiating events are widely believed to be independent of any immunogenicity possessed by the stimulant. These features of polyclonal responses provide the rationale for the use of such systems both as a model for the study of activation processes in lymphocytes²

TABLE 1 Cell surface markers and cell populations used

	T lymphocyte markers*		B lymphocyte markers*		EAC (C3)†
	anti-T sera‡	SRFC§ (E)	anti-B sera‡	anti-Ig	
1 Tonsil**					
Unseparated	52.8 ± 1.9	54.8 ± 1.6	44.5 ± 2.3	43.2 ± 1.8	34.0 ± 2.3
T cells: single cycle††	95(4)	94 ± 0.8	1.8 (4)	2.1 ± 0.3	0.2(2)
B cells: single cycle	1.3(3)	1.9 ± 0.3	97.1(4)	96.7 ± 1.5	86.4(3)
double cycle‡	—	<0.06	—	96.8	—
2 Spleen**					
Unseparated	—	32.2(4)	—	—	—
B cells: double cycle‡	—	<0.05	—	—	—

* Cell surface markers for human T and B lymphocytes reviewed in refs 17 and 18. Detailed methods for these markers are published elsewhere^{19,20,30}. Values given represent mean ± standard error of five to twenty-seven determinations. Mean value only given where number of observations (in parentheses) was less than five—means not tested.

† Rabbit anti-human brain serum absorbed with chronic lymphocytic leukaemia (CLL) cells.

‡ Rabbit anti-CLL cell serum absorbed with thymocytes³⁰.

§ Spontaneous rosette formation at room temperature with sheep erythrocytes (<2-week-old).

|| Rabbit anti-human immunoglobulin (polyspecific).

†† Rosette-forming cells with receptor for C3. Sheep red cells + rat IgM anti-sheep red cell antibody + mouse serum (complement). Test performed on a rotatory shaker at 37°C.

** Tonsils were obtained from patients undergoing tonsillectomy for recurrent throat infections. Spleens used in these experiments were obtained from accident cases. Single cell suspensions prepared using an Ultra-Turrax homogeniser followed by filtration directly through glass wool at room temperature to remove aggregates of cells.

††† T cells purified by filtration of T and B cells through columns of nylon fibres (Fenwall Leukopak)^{19,21,32}. Optimal conditions are described elsewhere¹⁹. Yields of T cells were 49 ± 2.9 (s.e.)%. T cell suspensions contained less than 1% cells capable of phagocytosing polystyrene particles in 50% foetal calf serum at 37°C.

‡‡ B cells were purified by removing those (T) cells forming spontaneous (E) rosettes by density centrifugation in Ficoll-isopaque¹⁹. Re-rosetting and a second separation (double cycle) was necessary to reduce T cell contamination to less than 0.1%. B cell yields were 62–83%. The proportions of phagocytic cells was not routinely assessed in these populations but is likely to be somewhat enriched at least with respect to monocytes, compared with the initial cell suspensions. Tonsil cell suspension contained 0.5% to 2% phagocytic cells and spleen cells 8% on the single occasion that they were assayed. Blood monocytes purified by the method of Huber and Fudenberg³³ were unreactive with all antisera used and did not form spontaneous rosettes. In a single experiment they did not form rosettes with EAC (mouse).

and clinically for the assessment of proliferative potential of blood-borne lymphocytes⁴.

Both of these aims have been facilitated by the finding that T and B lymphocytes in mice respond selectively to all mitogens so far tested with the exception of pokeweed mitogen (PWM), which stimulates both T and B cells^{5,6} and may in fact contain separate T and B mitogens. (F. Loo, personal communication). This selectivity is most clearly observed with lectins (such as phytohaemagglutinin (PHA) and concanavalin A (conA) and bacterial lipopolysaccharides which activate T and B cells respectively^{1–3}. Most polyclonal mitogens do however seem to bind equally well to both 'responder' and 'non-responder' cell types^{7–9}, and under appropriate circumstances (for example, insolubilisation of lectins^{10–11}) responses can be induced in a normally unresponsive population.

These convenient relationships are challenged by recent observations; the first, that some mouse^{1,12} and human¹³ B lymphocytes do respond to T mitogens in the presence of activated T cells, and second that human tonsillar B lymphocytes purified by a digestible immunoabsorbent method respond directly to PHA^{13,14}. The latter observation contradicts earlier circumstantial but nevertheless compelling evidence from studies on patients with selective immune-deficiency diseases which suggested that PHA was selective for T cells in man¹⁵. Indeed on the basis of these results and other data from mice, rats and chickens¹⁶ the proliferative response to PHA has been widely used to assess T cell status in man⁴.

We have investigated this problem by the application of a series of cell surface markers^{17,18} which readily distinguish T and B cells in man (Table 1). The T or B cell origin of activated lymphoblasts has been assessed in cultures containing T plus B cells, B cells only or T cells only. Purification of T and B lymphocytes was carried out by negative selection: T cells were eliminated by density sedimentation of cells which had formed 'spontaneous' rosettes with sheep erythrocytes and B cells removed by adherence to nylon fibres¹⁹. Lymphocytes from tonsils, spleen and blood have been extensively studied with different culture conditions, media and mitogen concentrations.

Figure 1 illustrates representative results using optimal doses of PHA and PWM to stimulate tonsil and spleen lymphocytes. Tonsillar T cells responded well to both PHA and PWM. B lymphocytes were not activated by PHA and gave only a small response with PWM. In cultures containing both T and B cells, T lymphoblasts predominated in the response to both PHA and PWM. But up to 15% of the PHA-activated lymphoblasts in tonsil cells cultures with either Dulbecco's or RPMI medium on day 3 may be B cells. Repeat experiments on tonsils and also with blood lymphocytes have given consistent results. The response profile of spleen cells in RPMI medium was different from that of tonsils. T lymphoblasts again predominated the PHA response of unseparated cells (85%); however the majority (88%) of lymphoblasts in PWM stimulated cultures were B cells. Purified B lymphocytes responded well to PWM but as with tonsil B cells no activation was detectable with PHA. Weak but significant spleen B lymphocyte responses have also been induced by *Escherichia coli* lipopolysaccharide (LPS) and staphylococcal enterotoxin B. (unpublished observations of G. J. and M. F. G.).

These results with human lymphocytes parallel our earlier observations of the cell types responding to PHA and PWM in mice², but they contradict those of Phillips *et al.*^{13,14}. While it can be agreed that the response to PHA of mixed T plus B lymphocyte cultures may involve a small and perhaps variable B cell component we could not confirm the results suggesting that purified B cells respond to PHA independently of T cells. The failure of purified human B lymphocytes to respond to PHA has also recently been found when cell purification was achieved by using cytotoxic anti-T lymphocyte sera (refs 20 and 21, and M. Cooper, personal communication). In addition there is a considerable body of clinical data which illustrates clearly that lymphocytes from patients with thymic aplasia which are almost entirely B cells do not respond to PHA^{22,23}. Responsiveness can however be instated by T cell reconstitution following thymus grafting²².

Despite this compelling weight of evidence the positive result of Phillips and Roitt cannot be ignored and demands an explanation. If we assume that the discrepancies do not

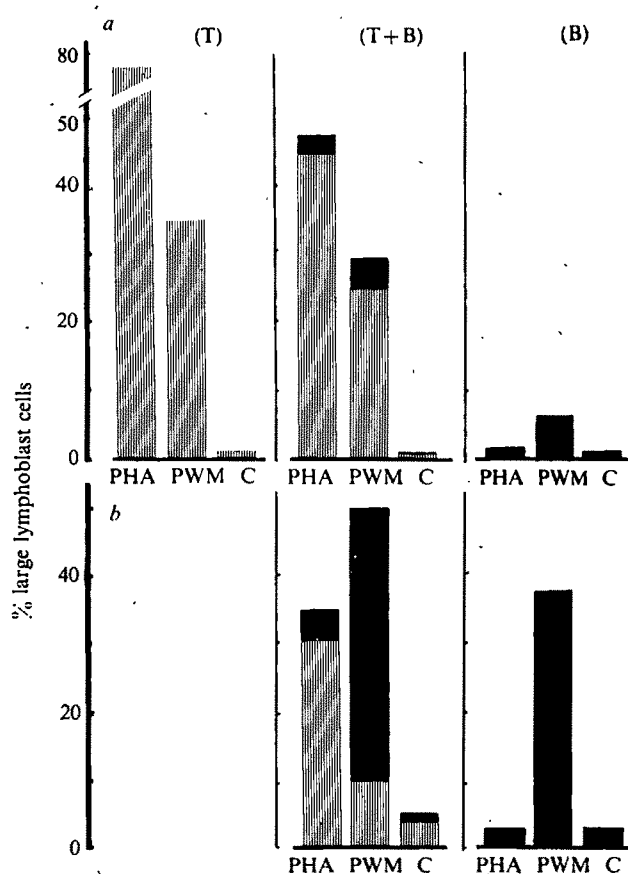


FIG. 1 Response of human T and B lymphocytes to phytohaemagglutinin and pokeweed mitogen *in vitro*. □, Lymphoblasts positive for T cell surface markers. ■, Lymphoblasts positive for B cell surface markers. a, Tonsil; b, spleen. The culture methodology was essentially as described previously^{18,28}. Here tonsil cells were cultured in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated human AB serum, and spleen cells in RPMI 1640 with 10% heat inactivated foetal calf serum. PHA was the purified preparation from Burroughs Wellcome and the responses shown are with $2 \mu\text{g ml}^{-1}$ assayed on day 3. PWM has protein derivative of *Phytolacca americana* prepared by TCA precipitation of a crude saline homogenate of stems and roots. The responses shown are with $10 \mu\text{g ml}^{-1}$ on day 3. The proportions of T and B cells in the cultures at 0 h were within the range indicated in Table 1. T lymphoblasts were typed as (S-RFC⁺, Ig⁺, B-Ag⁺) and B lymphoblasts as (S-RFC⁺, Ig⁺, B-Ag⁺), see Table 1. In additional experiments presumptive T and B lymphoblasts were also typed with EAC, anti-T cell serum and an anti-T lymphoblast serum analogous to that described by Phillips and Thomas¹⁴. Results with these markers paralleled very precisely those with the three markers used routinely. Aggregates of lymphoblasts were disrupted by vigorous pipetting and in occasional experiments with an Ultra-Turrax homogeniser. Rosetted suspensions were stained with 0.1% toluidene blue and viewed under ordinary light. Binding of anti-T, anti-B and anti-Ig sera to lymphoblasts in suspension was assayed using fluorescein-labelled goat anti-rabbit immunoglobulin and viewed with a Vickers M41 Photoplan fluorescence microscope with incident illumination. In all assays only cells with diameters greater than $10 \mu\text{m}$ were scored as lymphoblasts. Analysis of May-Grunwald/Giemsa stained smears confirmed that over 95% of enlarged cells were indeed lymphoblasts. Absolute numbers of lymphoblasts were counted and the response expressed as the number of lymphoblasts in relation to the initial cell number in the culture.

relate to quantitative problems or mistaken cellular identity then the following possibilities seem to us as the only plausible explanations for the discrepancy: our methodology may be inadequate for B cell responses to PHA either because culture conditions themselves are suboptimal and biased towards T cells, or alternatively because we have either lost or suppressed potentially responsive B cells during our purification procedure. We suspect this explanation is

unlikely to be correct since our B cell cultures do respond to PWM by both morphological transformation and DNA synthesis (30% of PWM-stimulated B lymphoblasts incorporate thymidine between days 3 and 4; unpublished observations of G. J. and M. F. G.). Moreover our culture conditions and media do not differ in any obviously significant way from those of Phillips and colleagues (ref. 13 and personal communication from Roitt and Thomas). Clearly however it is not possible at present to entirely rule out this explanation.

Alternatively it may be that the B lymphocyte responses to PHA observed by Phillips *et al.* are dependent upon facilitation mechanisms absent in our and others' systems. Potential factors of significance include the presence of macrophages, small numbers of activated T cells and subliminal stimulation by anti-immunoglobulin antibodies that are released during B cell purification on dextranase digestible Sephadex immunoabsorbent. Evidence can be procured to support all three possibilities. Macrophages have been found to potentiate PHA responses of both T cells²⁴ and B cell enriched cultures (L. Epstein, W. Kreth and L. Herzenberg, personal communication) and our own results indicate that contamination of B cells with as few as 2% T cells facilitates a PHA response. But this T-cell dependent response is also expressed principally by the 'residual' T cell (unpublished observations). Nevertheless the additional finding that culture supernatants of conA-activated T cells facilitate a small B cell response to this mitogen²⁵ provides evidence for a T-cell dependent, B cell response. Facilitation of PHA activation by binding of anti-human immunoglobulin antibodies which by themselves may be non- or minimally mitogenic seems to be a tenable explanation since such synergistic effects have been observed between anti-immunoglobulin and antigen in mice²⁶ and different mitogens in man²⁷.

These latter three explanations have at least the virtue that they can be readily tested. Regardless of the eventual outcome of this issue two points emerge which are relevant to the clinical application of proliferative responses to PHA. Under defined conditions the PHA response can be T-cell dependent and expressed predominantly by T lymphocytes themselves. In our hands optimal T cell proliferation with minimal B cell involvement is observed when cells are cultured in Dulbecco's modified Eagle's medium with 10% human AB serum. The PHA response can therefore be used as a quantitative or semiquantitative assay for the proliferative response of blood-borne T cells. A small proportion (5–15%) of B cells may however become activated in T-B cell mixtures (that is, in most clinical situations) and the application of cell surface markers is required to delineate this B cell contribution.

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Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system

RECENT experiments¹⁻³ indicate that cooperation between thymus derived lymphocytes (T cells) and antibody-forming cell precursors (B cells) is restricted by the H-2 gene complex. Helper activity *in vivo* operates only when T cells and B cells share at least one set of H-2 antigenic specificities. Evidence is presented here that the interaction of cytotoxic T cells with other somatic cells budding⁴⁻⁵ lymphocytic choriomeningitis (LCM) virus is similarly restricted.

Both the cytotoxic assay used and the characteristics of the cells involved have been described previously⁶⁻⁸. Briefly, monolayers of C3H mouse fibroblasts (L cells) are grown in plastic tissue culture trays, infected with a high multiplicity of the WE3 strain of LCM virus and the cells labelled with ⁵¹Cr and overlaid (40:1) with the spleen cell preparation to be tested. Supernatants are removed between 15 and 16 h later and % ⁵¹Cr release calculated⁷. Results are expressed as mean \pm s.e.m. for four replicates. Cytolysis of

TABLE 1 Cytotoxic activity of spleen cells from various strains of mice injected i.c. 7 d previously with 300 LD₅₀* of WE3 LCM virus for monolayers of LCM-infected or normal C3H (H-2^k) mouse L cells.

Experiment	Mouse strain	H-2 type	% ⁵¹ Cr release†	
			Infected	Normal
1	CBA/H	k	65.1 \pm 3.3	17.2 \pm 0.7
	Balb/C	d	17.9 \pm 0.9	17.2 \pm 0.6
	C57Bl	b	22.7 \pm 1.4	19.8 \pm 0.9
	CBA/H \times C57Bl	k/b	56.1 \pm 0.5	16.7 \pm 0.3
	C57Bl \times Balb/C	b/d	24.8 \pm 2.4	19.8 \pm 0.9
	nu/+ or +/+		42.8 \pm 2.0	21.9 \pm 0.7
2	nu/nu		23.3 \pm 0.6	20.0 \pm 1.4
	CBA/H	k	85.5 \pm 3.1	20.9 \pm 1.2
	AKR	k	71.2 \pm 1.6	18.6 \pm 1.2
3	DBA/2	d	24.5 \pm 1.2	21.7 \pm 1.7
	CBA/H	k	77.9 \pm 2.7	25.7 \pm 1.3
	C3H/HeJ	k	77.8 \pm 0.8	24.5 \pm 1.5

* Other mice were injected with 2×10^6 LD₅₀, but levels of specific release were invariably lower due to the high dose immune paralysis^{8,20} associated with viscerotropic (WE3) LCM virus.

† % ⁵¹Cr release by normal spleen cells on infected targets ranged from: (experiment 1) 17.1 \pm 0.3 to 20.0 \pm 0.7; (experiment 2) 20.0 \pm 1.4 to 25.3 \pm 0.7; (experiment 3) 27.2 \pm 2.0.

infected L cells by CBA/H immune spleen cells has been shown to be a property of specifically sensitised thymus-derived lymphocytes, which act in the absence of both macrophages and substances secreted into the medium at large⁶⁻⁸.

Various strains of mice were injected intracerebrally (i.c.) with 300 mouse LD₅₀ of WE3 LCM virus. Mice were sampled 7 d later when, in CBA/H mice, maximal cytotoxic activity is found in lymphoid tissue^{6,7}. Only spleen preparations⁹ from mice sharing at least one set of H-2 antigenic specificities with the target monolayer caused high levels (40% to 50%) of specific lysis (Table 1). Spleen cells from nude control (nu/+ or +/+) mice, derived locally from CBA and Balb/C stock (Dr J. B. Smith, personal communication), were less active and lymphocytes from histoincompatible mice caused minimal specific release (< 5%) of ⁵¹Cr.

Spleen preparations from mice immunised 10 and 13 d previously were also assayed, as Marker and Volkert⁹ have reported that maximal cytotoxicity of C3H lymphocytes for L cells infected with the Traub strain of LCM virus occurs at 11 d after inoculation. High levels of specific ⁵¹Cr release were again recognised only in the histocompatible system (Table 2) activity declining, as has been shown previously⁷, from a peak on day 7.

Demonstration of reciprocal exclusion of cytolysis was essential to establish that mice possessing other than H-2* antigenic specificities are capable of generating cytotoxic T cells. Comparisons were thus made using similar targets from allogeneic mouse strains. Peritoneal macrophages were obtained¹⁰ from normal Balb/C and CBA/H mice, cultured in plastic tissue culture trays and infected¹¹ with WE3 LCM virus. Specific lysis was restricted to the syngeneic system

TABLE 2 % ⁵¹Cr release* from infected C3H L cells overlaid with spleen cells from mice sampled at 7, 10 and 13 d after intravenous inoculation with 2,000 LD₅₀ of WE3 LCM virus.

Mouse strain	Days after inoculation		
	7	10	13
CBA/H	72.0 \pm 2.0	66.4 \pm 1.4	27.5 \pm 0.5
Balb/C	26.1 \pm 0.7	28.0 \pm 1.6	22.7 \pm 1.8
C57Bl	27.3 \pm 1.1	24.3 \pm 1.8	24.0 \pm 0.4

* Levels of ⁵¹Cr release due to overlaying normal L cells with immune spleen cells, infected L cells with control spleen cells or with medium alone ranged from 17.1 \pm 0.4 to 24.0 \pm 1.4. Other mice were injected with 2×10^6 LD₅₀, but levels of specific release were invariably lower.

TABLE 3 % ^{51}Cr release from normal and infected peritoneal macrophages by spleen cells from control mice and from mice injected i.c. with 300 LD₅₀ of WE3 LCM virus 7 d previously.

Spleen cells		Macrophage source	% ^{51}Cr release from macrophages			
			Experiment 1		Experiment 2	
			Infected	Normal	Infected	Normal
Balb/C	Immune	Balb/C	61.8 \pm 4.2 ^c	27.6 \pm 1.9 ^e	77.5 \pm 4.2 ^d	47.0 \pm 3.5 ^d
	Anti- θ *		ND	ND	40.6 \pm 2.5 ^e	ND
	N ascitic*		ND	ND	90.0 \pm 2.7	ND
	Control		42.0 \pm 4.8 ^a	40.5 \pm 5.2 ^a	49.6 \pm 2.5	43.5 \pm 1.6
CBA/H	Immune		42.7 \pm 6.7 ^a	33.7 \pm 5.4 ^a	32.9 \pm 3.0 ^a	48.6 \pm 3.9 ^a
	Control		28.0 \pm 4.1	40.5 \pm 5.2	46.5 \pm 3.7	39.7 \pm 4.3
CBA/H	Immune	CBA/H	69.1 \pm 2.8 ^c	30.9 \pm 3.4 ^c	72.5 \pm 5.2 ^d	40.0 \pm 2.9 ^d
	Anti- θ		ND	ND	44.0 \pm 2.5 ^d	ND
	N ascitic		ND	ND	74.3 \pm 8.4	ND
	Control		34.2 \pm 1.1	35.1 \pm 3.7	46.5 \pm 3.6	44.4 \pm 6.2
Balb/C	Immune		46.2 \pm 3.3 ^a	30.4 \pm 3.8 ^b	44.0 \pm 2.9 ^a	41.0 \pm 2.4 ^a
	Control		34.9 \pm 5.7	33.7 \pm 5.6	40.5 \pm 2.5	41.0 \pm 2.4

* Treated with AKR anti- θ (C3H) ascitic fluid and guinea pig complement, or normal AKR ascitic fluid and guinea pig complement.
^a, ^b, ^c, ^d, ^e. Differences by Student's *t* test between values for immune spleen cells treated with anti- θ ascitic fluid or normal ascitic fluid, immune and control spleen cells overlaid on infected macrophages (infected column), or immune spleen cells overlaid on infected and normal macrophages (normal column). ^a, $P > 0.05$; ^b, $P < 0.05$; ^c, $P < 0.02$; ^d, $P < 0.01$; ^e, $P < 0.001$.
 ND, Not done.

(Table 3). Comparable levels of specific ^{51}Cr release from isologous infected macrophages were caused by Balb/C and CBA/H spleen cells (overlaid at 20:1) from mice infected at the same time with the same dose of LCM virus, whereas histoincompatible macrophages were not damaged. Lysis was completely abrogated by treatment with AKR anti- θ ascitic fluid and guinea pig complement, but not by normal AKR ascitic fluid and complement. Though levels of ^{51}Cr release from macrophages were more variable than for L cells, probably because of inconsistencies in target cell concentrations and the higher background of non-specific lysis, the effect was both highly significant and repeatable.

The ability of T cells to cause lysis across an allogeneic barrier, when lymphocytes are sensitised to antigens specified by the H-2 gene complex, is well established^{12,13}. This also applies to L cells (H-2^k), which are readily lysed by spleen cells from C57Bl (H-2^b) mice stimulated in mixed lymphocyte culture by CBA/H (H-2^k) but not by Balb/C (H-2^d) lymph node cells. Sufficiently close association for lysis to occur is possible if the T cells is sensitised to alloantigens present on the surface of the target. Interaction between immune lymphocytes and cells expressing antigens expressed by LCM virus is, however, apparently confined to a histocompatible system, perhaps because it is only in this situation that the necessary intimacy of contact is achieved.

This restriction may possibly be overcome by previous treatment of the target population with trypsin. Balb/C (H-2^d) immune spleen cells will lyse recently trypsinised L cells (H-2^k) infected with the E-350 strain of LCM virus, the effect being completely abrogated by treatment with a rabbit anti-mouse brain serum cytotoxic for T cells¹⁴. Our experiments with previously trypsinised WE3-infected L cells have, to date, given equivocal results because of the high background of non-specific ^{51}Cr release.

An alternative possibility that must be considered in LCM is that the process of virus maturation⁵⁻⁶ through the cell membrane causes changes in self components, which are recognised only within the syngeneic or semi-allogeneic system. There is ample evidence¹⁵⁻¹⁶, for instance, that concentrations of H-2 antigens in the cell membrane are decreased in cells productively infected with budding viruses. The cytotoxic T cell may thus be recognising altered self, the implication being that LCM is essentially on autoimmune phenomenon.

These results impose a possible constraint on attempts to demonstrate cytotoxic T cells in infections of man and domestic mammals, where histocompatible cell lines and inbred strains are not available. Perhaps isologous macrophages or lectin-transformed peripheral blood leukocytes may prove

suitable targets in at least some disease states. Restriction of cell-mediated cytotoxicity within a syngeneic or semiallogeneic system may prove a reliable index of T cell involvement in species where the θ marker is not available, lysis across this barrier indicating an antibody-associated process¹⁷⁻¹⁹.

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Production of colony-stimulating activity by human lymphocytes

THE clonal growth of human bone marrow cells *in vitro* requires the presence of a stimulatory substance(s) designated colony-stimulating activity (CSA)^{1,2}. Data available from various clinical and animal experiments suggest that CSA is, or is related to, a physiological controller of granulopoiesis^{3,4}. Although the precise biological role of CSA is still uncertain, considerable insights into leukopoietic mechanisms have been gained from the study of interactions of CSA and haemopoietic cells *in vitro*. Two sources of CSA have been identified that stimulate colony formation from human bone marrow: certain foetal tissues and peripheral blood leucocytes^{3,4}. The observation that CSA is produced by white cells led naturally to hypotheses concerning the feedback control of stem cells by mature leukocytes^{3,5}.

Within the past year, three groups of investigators have fractionated human buffy coat into its component leukocyte subpopulations and identified the monocyte as the principal source of CSA⁵⁻⁷. Monocyte-derived macrophages and the human alveolar macrophage also elaborate CSA, whereas neutrophils and unstimulated lymphocytes do not⁸. Recently, however, we examined the effect of mitogens on human lymphocytes and observed that lymphocyte blast transformation was associated with the elaboration of CSA. Lymphocyte-derived CSA stimulated the production of bone marrow colonies containing both granulocytes and mononuclear phagocytes. These observations suggest that granulopoiesis is influenced by humoral substances released from lymphocytes reacting to appropriate stimuli.

Pure populations of neutrophils, monocytes and lymphocytes were separated from peripheral blood as previously described⁵: monocytes and PMN were separated by Ficoll-Hypaque gradients and glass adherence, lymphocytes by removal of other cells adherent to nylon fibre columns. As judged by morphology, peroxidase and alpha-naphthyl butyrase cytochemistry, and lack of phagocytosis, the lymphocyte populations were more than 98.5% pure. Contaminating monocytes were less than 0.1% of the populations—a number insufficient to account for the observed results. Rare nucleated erythrocytes and damaged neutrophils were observed as contaminants. Pure populations of monocytes, neutrophils or lymphocytes were cultured in Leighton tubes at 1.5×10^6 ml⁻¹ in McCoy's medium containing 2 or 15% foetal calf serum and either phytohaemagglutinin-P (PHA, Difco, 35 µg ml⁻¹), pokeweed mitogen (Grand Island Biological Co., 1:100 dilution), or saline. After 2-6 d the cell-free conditioned medium was collected and tested for CSA after incorporation of 0.05 or 0.1 ml into 0.5% agar underlayers. Normal human bone marrow was tested for its response to this conditioned medium by incorporating 2×10^6 cells in 0.3% overlayers^{1,5}. After incubation at 37° C for 8-10 d the colonies were counted, smeared and stained for morphological analysis.

As previously described^{5,8}, conditioned medium from monocyte preparations had CSA, whereas conditioned medium from unstimulated lymphocytes had no detectable activity. However, incubation of lymphocytes with PHA resulted in detectable stimulatory activity after 3-6 d. The results with pokeweed mitogen were variable until day 6, when low levels of CSA were consistently detected. Table 1 summarizes the results of one experiment which was repeated seven times.

PHA stimulated neither the production or release of CSA by neutrophils nor enhanced monocyte-related CSA. When lymphocytes were incubated in the absence of mitogen and the resultant conditioned medium was later combined with PHA or pokeweed mitogen, no detectable activity resulted. Neither PHA nor pokeweed mitogen—when incorporated directly in agar underlayers—had a detectable direct effect

TABLE 1 CSA activity of conditioned medium from cell cultures.

Cell type	Duration of culture (d)	Addition	Colonies per 2×10^6 marrow cells*
Neutrophils	2-6	Saline	0-2
Neutrophils	2-6	PHA	0-2
Monocytes	2	Saline	75 ± 7
	5	Saline	72 ± 6
	5	PHA	78 ± 8
Lymphocytes	2-6	Saline	0-2
	3	PHA	15 ± 3
	4	PHA	21 ± 3
	6	PHA	34 ± 5
	3	PWM	4 ± 3
	6	PWM	11 ± 3

PWM, pokeweed nitrogen.

* 0.05 ml of conditioned medium was incorporated in a 0.5 per cent agar underlayer, and 2×10^6 normal human bone marrow cells in a 0.3% agar overlayer.

on bone marrow growth. When lymphocytes were incorporated directly in agar with mitogen, many small cell clusters resulted in the underlayer, which obscured evaluation of growth of bone marrow colonies in the upper layer.

The time course of production of lymphocyte CSA in response to PHA is shown in Table 1. Activity was not detectable before day 2, but then increased progressively until day 6. It was detectable when cells were cultivated in 2% foetal calf serum, as well as in the more usual 15%. Both neutrophilic and mononuclear cell colonies developed in bone marrows stimulated by lymphocyte conditioned medium. Occasionally, predominantly eosinophilic colonies were observed (less than 15% of colonies).

Several lines of evidence support the concept that all mammalian haemopoietic cells ultimately arise from a common pluripotent stem cell pool^{4,9}. Relatively recent evidence indicates that this is true for lymphoid, as well as non-lymphoid cell lines¹⁰. The factors controlling differentiation into one or another cell line are poorly understood but almost certainly involve short-range microenvironmental factors as well as systemic hormonal mechanisms¹¹. Granulopoiesis and mononuclear phagocyte production *in vivo* are influenced by many stimuli, including bacterial products, antigens and leucocyte removal and administration^{8,12-14}.

The data reported here indicate that with mitogen-induced blast transformation, lymphocytes can elaborate a substance(s) able to stimulate the formation of granulocyte and mononuclear cell colonies from human bone marrow *in vitro*. These observations may explain the high levels of CSA observed in mice after antigenic stimulation¹⁴, and may serve to explain enhanced granulopoietic activity in certain types of humoral and cellular immune reactions. It is not yet known whether lymphocyte CSA is identical to that produced by mononuclear phagocytes. The observation of eosinophilic colonies arising under the stimulus of lymphocyte CSA may provide insights into the intriguing relationship between lymphocytes and the development of an eosinophilic response^{15,16}.

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In vivo localisation of radiolabelled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice

SEVERAL attempts have been made to show the specific localisation *in vivo* of anti-tumour antibodies. Most of these studies, however, either in experimental animals^{1,2} or in humans³ were performed with antibodies obtained by adsorption and elution from poorly characterised crude tumour fractions.

Here we report the specific localisation of ¹²⁵I-labelled antibodies against carcinoembryonic antigen (CEA) in heterografts of human colon carcinomas growing in nude mice and show that they can be detected by external scintillation scanning. This model system enables us to test the ability of antibodies directed against a well-characterised human tumour-associated antigen to concentrate in human tumours without performing preliminary experiments in patients.

We have recently shown⁴ that CEA as identified by Gold and Freedman⁵ was present in heterografts of human colon carcinomas serially transplanted into nude mice (Balb/c

Nu-Nu from G1. Bomholtgard Ltd., Ry, Denmark). CEA was demonstrated on cryostat tumour sections by indirect immunofluorescence using a highly specific rabbit anti-CEA antiserum⁶. The histology of the grafted tumour as well as the localisation of CEA in this tissue was identical to that observed in the primary tumour. Also, CEA extracted from tumour grafts by the perchloric acid method⁷ was found by radioimmunoassay^{8,9} to be immunologically identical to reference human CEA⁶ and, moreover, to be present in similar concentrations in both heterotransplanted and primary tumours. Using a double antibody radioimmunoassay^{10,11}, it was possible to detect circulating CEA levels of 20-70 ng ml⁻¹ in sera of nude mice bearing colon carcinoma tumours larger than 1 g. No CEA was detected in sera of nude mice bearing colon carcinomas smaller than 0.5 g or bearing other human tumours, including breast carcinomas, hypernephromas and melanomas.

Anti-CEA antibodies were isolated by adsorption-elution from a specific immuno-adsorbent prepared in the following way. Eight milligrammes of purified CEA⁶ were polymerised with 200 mg of BSA at pH 4.8, using 2 ml of 1% glutaraldehyde solution¹². Following homogenisation, the polymer was mixed with an equal volume of Sephadex G-25 and packed in a 1 cm diameter column. One ml of hyperimmune goat anti-CEA antiserum which exhibited little reactivity with the non-specific cross-reacting antigen (NCA)¹³⁻¹⁵, was run through the column at a flow rate of 3 ml h⁻¹. The adsorbed antibodies were eluted with 3 M KSCN¹⁶, dialysed against 0.01 M Tris-HCl buffer, pH 7.5, and concentrated to 1 ml. This antibody fraction contained 2 mg of protein and was able to bind 50% of 1 ng of CEA at a dilution of 1:70,000 when titrated in the radioimmunoassay.

A hundred microgrammes of the antibody fraction were labelled with 2 mCi of ¹²⁵I using the lactoperoxidase method¹⁷, mixed with 1 ml of normal goat serum and filtered on a Sephadex G-200 column. The 7S antibody fraction was collected and used immediately for the localisation experiments. About 60-70% of the radioactive material in the 7S fraction was specifically bound to the CEA immuno-adsorbent. For control experiments, normal goat 7S globulins, treated with 3 M KSCN, were labelled with ¹²⁵I and further purified on Sephadex G-200.

Volumes of 0.2 ml of the 7S fraction containing about 2 µg of radioactive antibody (specific activity: 8 µCi µg⁻¹) and

Fig. 1 *a*, Nude mouse bearing a heterograft of human colon carcinoma is shown in the scanning position. *b*, The total-body scan from the same mouse obtained 3 d after injection of 2 µg ¹²⁵I labelled anti-carcinoembryonic antibodies (dose of radioactivity injected = 16 µCi).

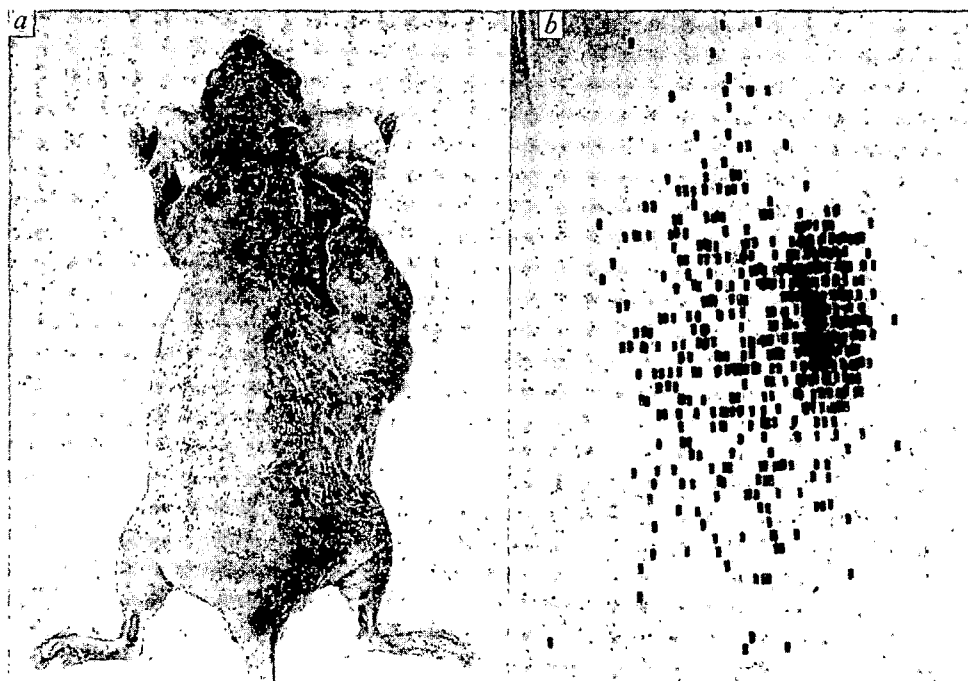


TABLE 1 Comparison of antibody and normal 7S concentration in tumour liver and muscles

Type and weight of tumours	Protein Injected	Tumour % per g*	Liver % per g*	Muscle % per g*	Tumour: liver ratio†	Specificity index†	Tumour: muscle ratio†	Specificity index†
Exp. I	Antibody	17.3	4.9	2.0	3.5	4.1	8.7	4.5
Co 115 1.620 g	N 7 S	4.6	5.3	2.4	0.9		1.9	
Exp. II	Antibody	24.5	4.4	1.2	5.6	2.7	20.4	2.4
Co 115 1.960 g	N 7 S	13.4	6.4	1.6	2.1		8.4	
Exp. III	Antibody	23.4	2.6	2.8	9.0	3.9	8.4	4.1
Co 115 2.200 g	N 7 S	13.2	5.7	6.5	2.3		2.0	
Exp. IV	Antibody	24.3	6.9	7.2	3.5	3.5	3.4	3.5
Co 115 0.575 g	N 7 S	12.4	12.4	12.8	1.0		1.0	
Exp. V	Antibody	15.0	10.0	1.8	1.5	1.9	8.3	1.8
Co 111 0.495 g	N 7 S	7.6	9.4	1.6	0.8		4.8	
Exp. VI	Antibody	12.9	6.3	1.7	2.0	2.3	7.6	2.1
Co 112 0.405 g	N 7 S	8.5	9.9	2.3	0.9		3.7	
Exp. VII	Antibody	7.4	3.9	1.8	1.9	1.2	4.1	1.0
El 4 0.510 g	N 7 S	8.9	5.5	2.1	1.6		4.2	

* Concentration of either antibody or normal 7S globulin (N 7S) expressed as % of the total specific radioactivity per g of tissue.

† Tumour: liver or tumour: muscle ratio are obtained by dividing the tumour concentration of either antibody or normal 7S globulin by the organ concentration of each of these proteins.

‡ Specificity indices are obtained by dividing the tumour: normal organ concentration ratio of antibody by that of normal 7S globulin.

200 µg of normal goat 7S globulins were injected intravenously into nude mice bearing subcutaneous grafts of human colon carcinomas. At different time intervals ranging from 2 h up to 3 d after injection, mice were scanned with a 3 inch crystal photoscanner (Picker Magnascanner 500). During the scanning procedure, the mice, anaesthetised by intraperitoneal injection of phenobarbital, were immobilised in the prone position as shown in Fig. 1a. After 2–6 h, the radioactivity was homogeneously distributed throughout the mice with no apparent localisation in the tumours. No difference could be detected between tumour-bearing mice and normal controls. After 1 d, however, the radioactivity began to localise in the tumour area and gave an optimal detectable contrast by day 3 (Fig. 1b). At that time, the mice were killed and scanned again after separating the tumour from the body. This last scanning confirmed that the major radioactive site was related to the tumour and not to the adjacent liver.

After complete dissection of the animal, each organ and pieces of the body were weighed and placed in well-type scintillation counter. For the 24 g mouse seen in Fig. 1, it was found that 40% of the total radioactivity recovered was concentrated within the 1.8 g tumour. Several other scanning pictures showing clear tumour localisation were obtained with different mice bearing variously sized heterografts, all derived from the same colon carcinoma (Co 115). The smallest tumour nodule detectable by scanning weighed 200 mg. Grafts of two other human colon carcinomas (Co 111 and Co 112), with more differentiated histology and less abundant stroma and vascularisation⁴ than Co 115, showed a lower specific concentration of antibodies (see Table 1) which were not detectable by scanning.

To take into account the possible non-specific accumulation of proteins in the extravascular space and necrotic regions of the tumour¹⁸, the antibody localisation was studied by simultaneous injection of ¹³¹I-labelled antibodies and ¹²⁵I-labelled normal goat 7S globulins (N 7S). In two experiments, the labels were reversed. The average values of four representative experiments performed in mice bearing Co 115 tumour are presented in Fig. 2. The results are expressed in percentage of the total radioactivity recovered per g of each tissue studied. The concentration of antibodies in the tumour was more than two-fold greater than N 7S whereas in normal tissues the antibodies had lower values than the control protein.

In Table 1 the individual results from seven different experiments are summarised. Concentration values of labelled antibodies and N 7S observed in tumour, liver and muscle

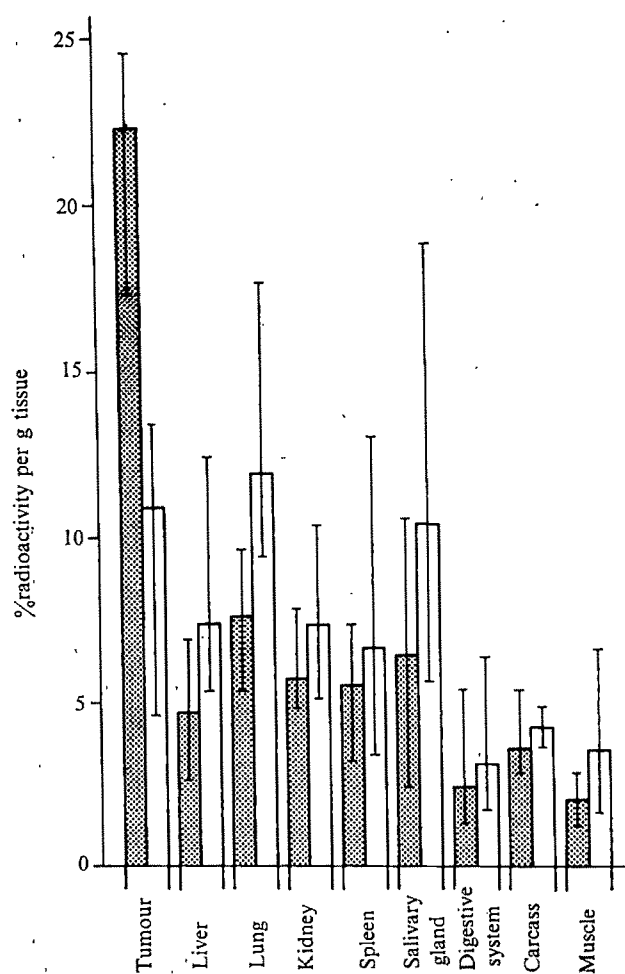


FIG. 2 Distribution of goat anti-CEA antibodies and normal goat 7S globulins in nude mice bearing heterografts of human colon carcinoma (Co 115). Three days after simultaneous injection of both proteins each labelled with a different iodine radioisotope (as described in the text), the tumour and different tissues were weighed and then measured in a dual channel well-type scintillation counter. Bar graphs represent the average value of four different experiments (% of total specific radioactivity recovered per g of tissue) for antibodies (shaded area) and for normal goat 7S globulin (open area). The solid lines represent the range of values.

can be compared. For both radioactive preparations, the ratio of tumour:normal organ concentration was calculated. A specificity index was obtained by dividing the concentration ratio of antibodies by that of N 7S. This index was significantly positive in all colon carcinomas tested, including Co 111 and Co 112 (experiments V and VI), which were not detectable by scanning. In contrast, when the same experiments was performed in C57Bl/6 mice bearing a transplanted EL4 solid lymphoma (experiment VII), the specificity indices were not significant.

Evidence was obtained that the specificity of antibody localisation was decreased with the presence of necrotic tissue within the tumour. In experiment II, the tumour exhibited a significant degree of central necrosis. Despite a high absolute concentration of antibodies in this tumour, relatively low specificity indices were observed compared with those obtained for less necrotic tumours (experiments I, III and IV). Furthermore, when the radioactivity of a small non-necrotic fragment selected from the tumour of experiment II was measured, the tumour:liver ratio of antibody was 14.0 and the specificity index reached 8.6 (the highest value yet observed). In three other experiments, cells from disrupted tumour fragments were counted after several washes with buffered saline. It was found that about 55% of the antibody radioactivity remained bound to the cells compared with 15% of the N 7S radioactivity.

It should not be concluded from these experimental studies that the injection of patients with labelled heteroanti-CEA antibodies will, in any case, enable the scanning detection of CEA-containing cancers. The major difficulties in humans would be the relatively large amounts of circulating CEA^{8,9,11}, as well as the presence of small quantities of CEA in non-malignant tissues^{6,19-21}. Further work is needed to assess whether the use of highly specific antibodies directed against CEA may open a new dimension in the field of tumour localisation by radioactive tracers.

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Immunological detection of antigen(s) associated with rat colon carcinoma

CANCER of the colon and rectum is exceeded only by cancer of the lung as a cause of cancer death in the United States. Of the approximately 350,000 deaths from cancer in 1973, 47,400 were from cancer of the colon and rectum; of the approximately 665,000 cancers diagnosed in 1973, 79,000 were in the colon and rectum. Denmark, Great Britain, Ireland, Canada, New Zealand, Australia, Belgium and Austria have equally high or even slightly higher death rates from this disease and it is a significant cause of morbidity and mortality in most western countries. Improved surgical techniques and the development of new diagnostic methods have not significantly affected these mortality statistics¹.

A useful animal model for the study of human colon carcinoma is the rat bearing adenocarcinomas of the colon induced by 1,2-dimethylhydrazine (DMH)^{2,3}. Six to 8 months after initiation of treatment, 60 to 100 per cent of rats have one or more colon tumours which cause bleeding, intussusception or obstruction. The tumours are visible through the thin wall of the colon and are readily accessible for surgical or other treatment. Methods for early detection of tumours are needed for (a) development and evaluation of preventive or therapeutic measures and (b) studies of tumour development under dietary or other regimens designed to retard or enhance colon carcinogenesis³⁻⁵.

Human colon carcinomas produce a glycoprotein, carcinoembryonic antigen (CEA)⁶, which is detectable in serum⁷ or plasma⁸. Although CEA is not specific for colon tumours, detection and quantitation of circulating quantities may be useful in the diagnosis of colon and other cancers, for prognosis after surgical resection and for monitoring chemotherapy⁹. We have detected antigen(s) in DMH-induced rat colon carcinomas using antisera produced in rabbits (see below). Since the antigen(s) was found in the rat tumours but not in normal rat colon it may provide a rat model for CEA in human colon carcinoma.

Identification of the tumour-associated antigen analogous to human CEA, in the rat model described above would facilitate study of (1) the relationship between the blood level of antigen and size or other anatomic characteristics of colon tumours, (2) response of tumours to treatment, and (3) experimental induction and development of tumours.

Twenty-eight male, 4-week-old Sprague-Dawley rats (Charles River Laboratories) were fed a semisynthetic diet for 3 months, given ten weekly intragastric doses of DMH (30 mg kg⁻¹ in 0.9% NaCl) and killed when they developed signs of tumour in the intestine or ear duct. Then control rats were given 0.9% NaCl. At autopsy, colon tumours 3

mm or greater in diameter and sections of normal colon (starting 1 cm away from the tumour) were frozen in liquid nitrogen. A small section of tumour was fixed in 10% neutral buffered formalin and processed for histological examination.

Pooled tumours (4.2 g) were minced and homogenized for 1 h at 0° C in three volumes of 0.9% NaCl using a motor-driven Teflon pestle. The homogenate was centrifuged at 500 g and 4° C for 30 min; the supernatant was dialysed against distilled water for 60 h with fifteen 4-h changes. The non-dialysable portion was flash frozen in methanol-dry ice and lyophilized to dryness to yield 186 mg of product. Extracts were prepared, by the same procedure, from 4.9 g of normal colon from ten control rats and 5.3 g of normal colon from tumour-bearing rats. Portions of the three extracts were dissolved separately in 0.9% NaCl at a concentration of 4 mg ml⁻¹.

Antibodies to the tumour macromolecules were produced by giving New Zealand adult rabbits three weekly intramuscular injections of a mixture of 0.25 ml of tumour extract and 0.75 ml of aqueous aluminum hydroxide gel as an adjuvant¹⁰. The rabbits were bled 7 d after the last injection, injected with the tumour extract-aluminum hydroxide gel mixture and bled repeatedly for several weeks.

The blood was allowed to clot at room temperature for 1 h, held at 4° C overnight and centrifuged at 280 g and 4° C. Sera were decanted and centrifuged at 56° C for 30 min. Antibodies to tumour-associated antigens and normal rat tissue components were found in the antisera by double immunodiffusion (Ouchterlony) and counterimmunoelectrophoresis techniques on rehydratable Agarose media. Heterologous antibodies were removed from the antisera by absorbing with (a) extracts of normal rat colon (1 part to 100 parts of the antisera) and (b) affinity chromatography beads (CNBR-Sepharose, Pharmacia) to which normal rat colon macromolecules were coupled. Absorption was continued until the antisera did not react with normal colon components when assayed by the Ouchterlony technique. Two-fold serial dilutions of normal colon extracts were tested with the absorbed antisera to confirm that negative reactions were not due to formation of soluble immunocomplexes in the prozones of the antisera.

Absorbed antisera to rat colon tumours (10 µl) were reacted by the Ouchterlony technique with 10 µl of extracts of tumour, normal rat colon and normal colon from tumour-bearing rats for 48 h in a humid chamber at room temperature. Reaction was obtained only with extracts of tumour. Counterimmunoelectrophoresis was performed on prototype 35 mm Agarose strips (45 mA, 30 V, 60 min) in which macromolecules from the colon tumours and normal colon were electrophoretically transported to the absorbed tumour antibodies¹⁰. The same concentrations were used as in the Ouchterlony technique. This more sensitive technique supported the Ouchterlony results. When seven two-fold serial dilutions of colon tumour extract and of normal colon extract were reacted with a constant concentration of antibody to rat colon tumours, reactions were obtained with the tumour extract at all dilutions, but no reactions were obtained with dilutions of the normal colon extracts.

Preliminary work indicated that the absorbed antisera reacted with rat foetal extracts. Forty foetuses taken from four Sprague-Dawley rats on the fourteenth day of gestation were dissected free of maternal tissues, decapitated and their tails docked. Pooled foetuses gave 2.8 g of tissue from which a crude saline extract was prepared by homogenization and centrifugation. Counterimmunoelectrophoresis performed as described above indicated a strong reaction between foetal extract components and the tumour antibodies. Preliminary results indicate that the tumour antibodies do not cross-react with human CEA and that rat tumour antigen does not react with anti-CEA antisera. The difference may be one of species specificity as was found with α -foetoprotein produced by human or rat hepatocarcinomas¹¹.

A. B.-K. G. carried out this work in partial requirement for the MS degree.

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Involvement of amino and sulphydryl groups in olfactory transduction in silk moths

ROZENTAL and Norris¹ found evidence of a chemosensory mechanism of olfaction and gustation in the cockroach *Periplaneta americana* that involves the formation of disulphide bonds. I wish to report that olfactory transduction in silk moths does not seem to involve disulphide links, although sulphydryl and amino groups are important.

Experimental evidence on mechanisms of molecular interaction with chemoreceptors might aid the choice among different theories of taste and olfaction²⁻⁸, and the silk moths are useful models for such work because the antennae of the males contain cells specialised to capture chemical information from a particular molecular species⁹⁻¹⁵. I have perfused amputated male antennae of the silk moths *Antheraea pernyi* and *Bombyx mori* with solutions of chemical reagents selective for protein side chains. (Previous control perfusions were performed with the specific solvents for the reagents.) Olfactory responses were measured by the electroantennogram method^{9,14}. Unlike that of *B. mori*, the sex pheromone of *A. pernyi* is not yet available, and so I prepared an extract from the female glands¹².

2,3-Dimethylmaleic anhydride (DMA) and 2-methylmaleic anhydride (citraconic anhydride, CA) are specific blocking agents for amino groups of proteins¹⁶, but their action is reversible by decreased pH. Olfactory responses were inhibited with DMA, pH 7.5, at a threshold concentration of 2 mM. On re-perfusion with control Ringer solution, pH 4.8, there was substantial reversal of inhibition. Figure 1a shows the electroantennogram responses before, during and after the reversal of inhibition by 4.7 mM DMA. Similar reversible inhibition was obtained with CA. There was also inhibition with ≥ 0.9 mM 2,4,6-trinitrobenzenesulphonic acid¹⁷.

Behavioural and other studies have suggested that sulphhydryl groups are involved in taste and olfaction in various insects¹⁸⁻²⁰ and man²¹. To test for such involvement in *A. pernyi*, I perfused antennae with N-ethylmaleimide (NEM) at pH 7.5. Inhibitions graded according to concentration were found (Fig. 1b). *p*-Chloromercuribenzoate was inhibitory at a threshold of 0.2 mM; appreciable reversal was achieved by re-perfusion with 1.0 mM reduced glutathione. Mersalyl (salyrganic acid) and iodoacetic acid inhibited, with thresholds of approximately 1 mM and 0.1 mM respectively. I found similar, though less strong indications that amino and sulphhydryl groups are involved in the olfactory process of *B. mori*; the antennae are less robust in successive perfusions than the larger antennae of *A. pernyi*.

The exact site of action of an inhibitor on a whole antenna is unknown, and rather than the receptor macromolecule, ion-channels or an ion-pump mechanism, for example, might be

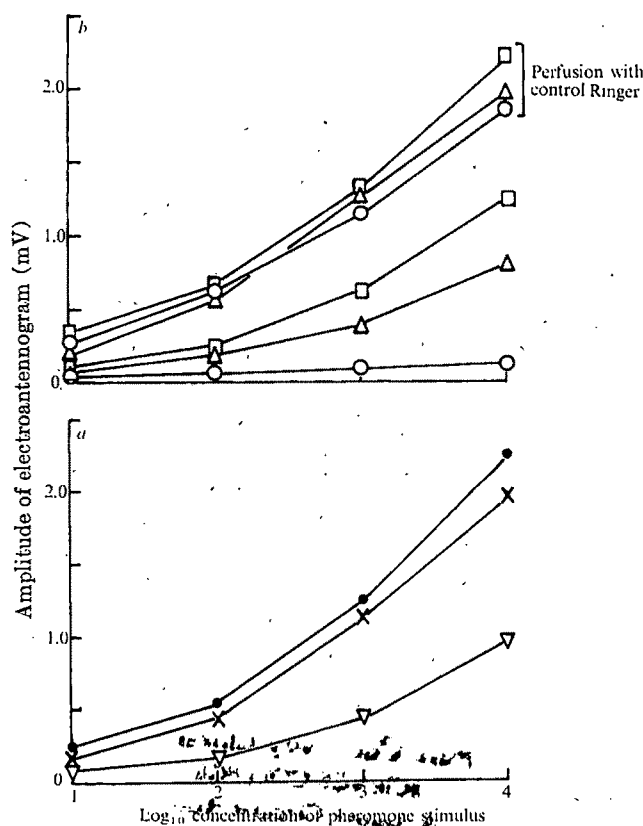


FIG. 1 Olfactory responses (amplitude of electroantennogram) of *A. pernyi* male antennae after perfusion with reagents for (a) amino and (b) sulphhydryl groups of proteins. Since the concentration of pheromone in the extract of pheromone gland (see text) is unknown, the concentration of the stimulus is expressed in arbitrary units. a: ●, Control; ▽, 4.7 mM DMA at pH 7.5; ×, reversal of inhibition by re-perfusion with control Ringer adjusted to pH 4.8. b, Perfusions with various concentrations of NEM: □, control, followed by 0.1 mM NEM; △, control, then 1.0 mM NEM; ○, control, then 10.0 mM NEM. Data represent mean values for three antennae; the experimental variability was approximately ± 0.1 mV.

impaired. The latter is less likely, for standing potentials²² that I measured tended to be maintained after perfusion with inhibitors. The unambiguous identification of both the inhibitor target site and the modified groups, however, requires isolation of the receptor and the inhibition products.

The relatively high concentration needed for inhibition could indicate non-selectivity of action for the amino and sulphhydryl group reagents. Since diminished olfactory responses in *A. pernyi* did not change appreciably for almost 1 h after perfusion with the inhibitors, equilibrium may have been reached. But if the resistance to diffusive flux were high, because of a hidden site of action for example, the variation of concentration gradient with time would not be appreciable, thus giving rise to quasi-stationary diffusion²³. If so, the concentration of inhibitor at the binding-sites would be somewhat lower than in the perfusing system.

The next question I considered was whether disulphide groups might be involved in silk moth chemosensory transduction as they seem to be in the function of vertebrate acetylcholine receptors²⁴⁻²⁷ and taste and olfaction in *P. americana*¹. Antennae of *A. pernyi* were perfused with reduced glutathione, L-cysteine, dithioerythritol (DTE)²⁸ and β -mercaptoethanol. Inhibition was found only at concentrations greater than approximately 10 mM, and it was not reversible, suggesting non-specificity of action. Below 1 mM there was no detectable effect; from 1 mM to 10 mM responses were slightly activated; with DTE there was some activation even at 30 mM. A possible explanation for this activation is that receptor sulphhydryl groups were protected by the reducing agents. Similar results were obtained with *B. mori*.

A tentative conclusion is that, in contrast to findings with *P. americana*, interconversion between -SH and S-S^{1,29} is not characteristic of olfactory transduction in *A. pernyi* and *B. mori*.

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The α 1-acute phase protein response in rats as a possible indicator of the relative smoking risks of different cigarettes

AN increase in the serum levels of the α 1-acute phase protein, indicative of local tissue damage¹ has been shown in rats exposed to cigarette smoke for periods of up to 30 d. The increases correlated well with the degree of damage to the respiratory system as evidenced by the pathological changes observed *post mortem* in the lungs (L.A.E., T.E.B., D. A. Darcy and J. P. O'Sullivan, unpublished).

The greatest increases were in the rats exposed to the smoke of cigarettes made of flue-cured tobacco, which are by far the most popular type of cigarettes smoked in Britain. Considerable increases in the α 1-protein were also seen in rats exposed to the smoke of cigarettes made mainly from air-cured, unfermented tobacco, a type popular in France, Spain and other European countries. Rats exposed to the smoke of cigarettes made of air-cured fermented cigar tobacco, however, showed only a slight increase in their serum α 1-acute

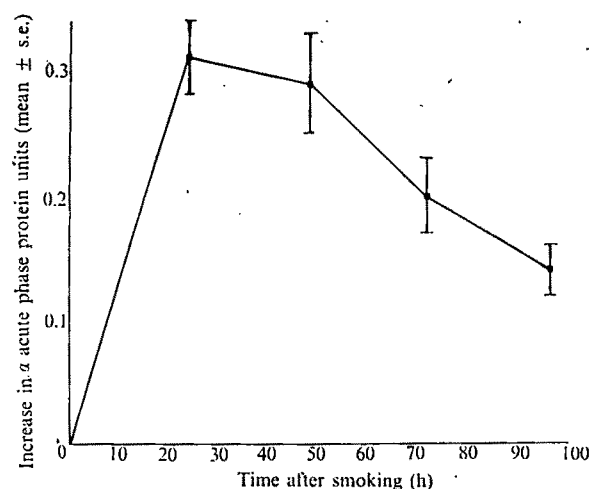


FIG. 1 The effect of smoking 35 cigarettes on rat serum α 1-protein.

α 1-Acute phase protein determinations were carried out by the method of Darcy^{2,3} on 0.2 ml blood taken from a tail vein. Groups of five rats were then exposed to the smoke from 30 to 40 cigarettes in a Martin Wright smoking machine⁴. The machine was set to give a 35 ml puff of 2 s duration and the smoking period lasted 3 to 4 h. The animals were again bled for α 1-acute phase determination at a fixed time, usually 24 h after the end of their smoking period. Male rats, of the Wilkie hooded strain, aged between 10 and 20 weeks bred under minimal disease conditions were used. Control animals were sham smoked.

Figure 1 shows the time response curve to the smoke of 35 commercial English cigarettes (made of flue-cured Virginia tobacco). The optimum response appears to be

TABLE 1 Effect of different tobacco smokes on serum α 1-acute phase protein in rats.

Tobacco type	Country	Sugar content (%)	pH of smoke	Tar T.P.M. (mg)	Nicotine (mg)	No. of cigarettes smoked	Increase in α 1-acute phase protein units (\pm s.e.)*
Flue-cured cigarette	England	17.8	4.0	21.8	2.2	40	1.10 \pm 0.06†
	England	17.8	4.0	21.8	2.2	35	0.97 \pm 0.09
	England	18.0	4.15	21.1	2.3	40	0.78 \pm 0.05
Air-cured cigarette	France	2.0	8.2	18.6	2.5	30	0.44 \pm 0.08
	France	2.0	8.2	18.6	2.5	30	0.29 \pm 0.01
	Switzerland	1.6	6.0	16.3	2.1	35	0.44 \pm 0.04
	Spain	0.5	8.6	17.5	2.6	40	0.27 \pm 0.05
Air-cured cigar	U. States	0.4	8.7	27.5	5.8	30	0.54 \pm 0.01
	U. States	0.4	8.7	27.5	5.8	30	0.24 \pm 0.02
Air-cured, fermented cigar	U. States	0.03	9.0	23.5	3.5	30	0.19 \pm 0.02
	U. States	0.03	9.0	23.5	3.5	30	0.05 \pm 0.01

Control rats not exposed to tobacco smoke

* Mean baseline for all rats 0.19 \pm 0.01.

† Mean of ten rats.

phase protein. This could be of significance in relation to the relatively low lung cancer mortality of cigar smokers compared to cigarette smokers. As the acute phase proteins usually appear in increased concentration in the blood within a few hours of inflammation or tissue injury, it seemed possible that the α 1-acute phase protein response in the rat could offer a convenient rapid method of comparing deleterious effects and possible smoking risks of different cigarettes. We report here preliminary observations.

between 24 and 48 h, and a 24-h interval between the end of the smoking period and determination of the α 1-acute phase protein response was selected for comparison of the effect of the smokers of different tobaccos shown in the table.

Although only limited inferences should be drawn from these preliminary results, the smoke of cigarettes made from flue-cured, high sugar content tobacco seems to produce the greatest increases in α 1-acute phase protein. Increases to between five and seven times the control (presmoking) levels

were observed. The smoke of air-cured tobacco cigarettes and of cigarettes made from unfermented cigar tobacco produced increases of up to 3.5 times the control value. The lowest increases, to less than twice the control values were found in animals exposed to the smoke of cigarettes made from air-cured fermented cigar tobacco. This agrees with the findings of our longer-term experiments, and is consistent with the much lower risk of lung cancer in cigar smokers compared with cigarette smokers.

The effect on the serum α 1-acute phase protein does not seem to bear any direct relation to the tar and nicotine content of the smoke. There may be a possibility that the acid nature (pH 4) of the smoke of the high sugar content, flue-cured tobacco⁵ makes some contribution to the intensity of the α 1-protein response.

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Synthetic insecticide with a new order of activity

We describe a new insecticide, related to the pyrethrins, and considerably more active than any other available compound, of whatever class (LD_{50} about 0.0003 μ g per insect, equivalent to about 0.03 mg kg^{-1}).

Recently, we synthesised insecticidal esters of 5-benzyl-3-furylmethyl alcohol¹ and 3-phenoxybenzyl alcohol^{2,3} with (1R, trans) 2,2-dimethyl-3-(2,2-dihalovinyl)cyclopropane carboxylic acids. (The nomenclature adopted here is a convenient alternative to that based on the sequence rule⁴ where, for example, the dihalovinyl acids formally derived from (+)-trans (1R, 3R) chrysanthemic acid by replacement of the vinyl methyl groups would be (1R, 3S)). The (\pm)-trans-dibromovinyl ester of 5-benzyl-3-furylmethyl alcohol has also been reported⁵. We have now prepared the corresponding (1R, cis) acids and esterified them with appropriate alcohols, including those of the α -ethynyl- (Belgian Patent 738112 to Badische Anilin-Soda-Fabrik Aktiengesellschaft and Ger. Offen. 2,230,862 to Sumitomo Chemical Company Limited) and α -cyanobenzyl (Ger. Offen. 2,231,312 to Sumitomo Chemical Company Limited) series. (1R, cis)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylic acid (Fig. 1b) was synthesised as shown via the aldehyde (Fig. 1a) derived from methyl (+)-cis (1R, 3S) chrysanthemate by an established method of ozonolysis (French Patent 1,580,474 to Roussel-Uclaf, S.A.). The ester (NRDC 156) with 3-phenoxybenzaldehyde⁶ cyanhydrin ((\pm)- α -cyano-3-phenoxybenzyl alcohol) (Fig. 1d) was very active as an insecticide. A solution of it in hexane deposited crystals after cooling for 2 months. Recrystallisation gave a single pure isomer (melting point 100°C, sharp; mass spectrometric molecular weight, 503; Br = 79) with an NMR spectrum showing only one signal for the CH(CN) proton of τ 3.64, whereas the parent mixture of isomers showed two signals of equal intensity at τ 3.64 and τ 3.72. The mother liquors contained material giving 2 signals (relative areas 1:4) also at τ 3.64 and τ 3.72.

TABLE 1 Toxicity of various insecticides to insects

Compound	Relative toxicity* to	
	Houseflies (<i>Musca domestica</i> L.)	Mustard beetles (<i>Phaedon cochleariae</i> Fab.)
Pyrethrin I	2.0	160
Bioresmethrin (NRDC 107)	100†	100‡
Cismethrin (NRDC 119)	41	52
K-othrin (RU 11,679)	130	170
Parathion	37	7
DDT	4-15	1.1
Dieldrin	35	4-10
(\pm)- α -cyano-3-phenoxybenzyl ester (NRDC 156)	1000	1000
NRDC 161 (crystalline component of above; see Fig. 1e)	2300	1600
Material in mother liquors from above crystallisation	350	380

* By topical application of acetone solutions as described previously¹, w/w basis.

† $LD_{50} \sim 0.006 \mu$ g per insect

‡ $LD_{50} \sim 0.005 \mu$ g per insect

Table 1 shows that the crystalline isomer (NRDC 161) was outstandingly active against insects compared with the non-crystalline form and with other insecticides. To mustard beetles, on a molar basis, NRDC 161 is twenty-four times as active as bioresmethrin^{7,8} (5-benzyl-3-furylmethyl (+)-trans-chrysanthemate, molecular weight 338) and fifteen times as active as pyrethrin I, the principal constituent of pyrethrum extract⁸. To a normal strain of houseflies NRDC 161 is thirty-four times as active per mol as bioresmethrin (itself more potent than most insecticides) and 1,700 times as active as pyrethrin I. The activity against houseflies was further enhanced by pretreatment (2 μ g per insect) with the synergist sesamex, which decreased the LD_{50} value from 0.00034 to 0.000018 μ g per insect (~ 0.002 mg kg^{-1}) (synergistic factor = 18, close to the value for less active synthetic pyrethroids⁹). The LD_{50} values for *A. stephensi* (ng per female) and *G. austeni* (ng per mixed sex) are respectively 0.036 and 0.08, giving an activity about forty-five times per mole that of bioresmethrin (F. Barlow, personal communication). The insecticidal activity of NRDC 161 and the effects of synergists are being studied further.

To obtain information about the absolute configuration of NRDC 161, (+)- α -cyano-3-phenoxybenzyl alcohol (optical purity, 95% from NMR spectrum of ester below) was prepared from 3-phenoxybenzaldehyde (Fig. 1c) and hydrogen cyanide in the presence of D-oxynitrilase¹⁰ (conveniently available in emulsion preparations, such as β -glucosidase, from Koch-Light Laboratories Ltd) and esterified with (1R, cis)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylic acid as in Fig. 1. The ester was not crystalline and resembled, in properties and insecticidal activity, material from the mother liquor after separating the crystalline isomer. If the (+)-cyanohydrin thus obtained from 3-phenoxybenzaldehyde has the same configuration as the D-(+)-mandelonitrile derived from benzaldehyde via the oxynitrilase¹⁰, the absolute configuration of the α -cyano-3-phenoxybenzyl alcohol in the potent crystalline isomer is S, for (+)-mandelonitrile is known to give (-)-mandelic acid, related to D-(+)-R-glyceraldehyde¹¹. The potent crystalline isomer, $[\alpha]_D + 15.6^\circ$, is therefore tentatively assigned the structure [S]- α -cyano-3-phenoxybenzyl cis-(1R,3R) 2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylate (Fig. 1e) pending determination of the absolute configuration by the X-ray examination (now in progress by Dr J. D. Owen and Dr M. R. Truter).

The discovery of a pyrethroid with such great toxicity to insects and atypical activity in mammals (see accompanying

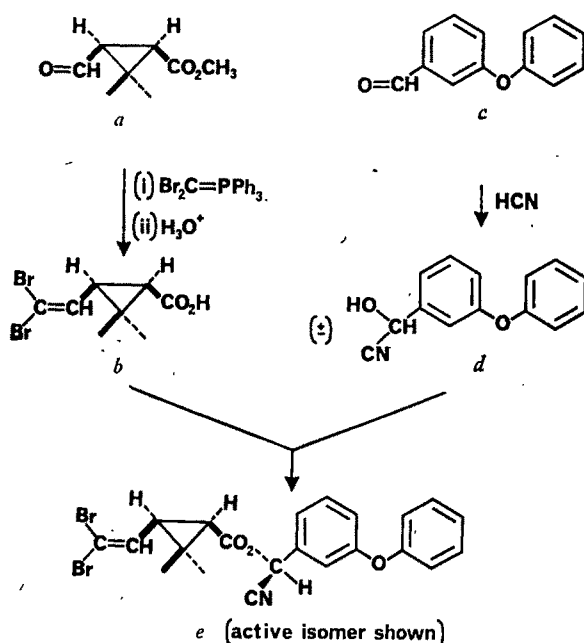


FIG. 1 Synthesis of the active compound.

paper¹²) has important theoretical and practical implications, now being examined.

The new compounds are protected by UK Patent Applications Nos 20539/73, 39539/73 and 49098/73 and corresponding foreign applications.

We thank Dr J. Martel (Roussel Uclaf SA) for a generous gift of (+)-cis-chrysanthemic acid, Dr J. M. Barnes, Mr R. D. Verschoyle and Mr F. Barlow for results and discussions, and the National Research Development Corporation for financial support.

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Toxicity of new pyrethroid insecticide

THE mammalian toxicity of NRDC 156 and NRDC 161 (see accompanying paper¹) was assessed in albino female, rats 10 to 12 week old.

Given as solutions in glycerol formal by the intravenous route, the lethal dose of NRDC 156 was 6 to 8 mg kg⁻¹ and of NRDC 161, 2 to 2.5 mg kg⁻¹. Orally, as solutions in arachis oil, an LD₅₀ could not be computed, but the rats showed severe toxic effects, with some deaths in the dose range 80 to 160 mg kg⁻¹ for 156 and 25 to 63 mg kg⁻¹ for 161.

The signs of poisoning in these rats were not the same as those described for other pyrethroids². The first sign was excessive salivation without lachrymation, rapidly followed by continuous irregular jerking movements of the limbs, progressing to rolling convulsions, and an occasional tonic-clonic convulsion. Death occurred from 12 min to 2 h after intravenous dosing and from 3½ to 28 h after oral dosing. Survivors were usually almost normal within 4 to 6 h after intravenous dosage, but recovery from an oral dose took up to 48 h.

The impression gained from observing the animals was that the site of action of NRDC 156 and 161 was mainly central, with little or none of the peripheral component that has been demonstrated for other pyrethroids.

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First frog fossils from Australia

RECENT speculation about the ancestral frog fauna of Australia, even embracing the Cretaceous¹, has been based purely on zoogeographic deductions, for study of the origins and history of this fauna has been seriously handicapped by the complete absence of an endemic fossil record. Although it has been suggested^{2,3} that the Lower Eocene *Indobatrachus* of the Intertrappean beds of Bombay represents the Australian leptodactylid subfamily Myobatrachinae, such a relationship is considered dubious or untenable by other authors^{4,5}. It is therefore of interest to report the recent discovery of isolated fragments of frog bones among a rich mid-Miocene vertebrate fauna in Central Australia, so being the first frog fossils found in Australia.

The material was obtained at Tedford Quarry, V-5375, on the west side of Lake Palankarinna in the extremely arid north of the State of South Australia. The site is in the Etadunna Formation, and the biological material recovered there is termed the Ngapakaldi Fauna by Stirton, Tedford and Woodburne⁶.

Most of the isolated anuran bones comprise limb fragments (particularly heads of humeri and femora) which, at present, cannot be referred to families, but there is included an almost complete left ilium (Fig. 1) which is 7 mm long and remarkably well preserved. This specimen is of particular value because of all isolated anuran bones the ilium is considered to be of the greatest diagnostic value at the familial, generic and even specific level^{7,8}. The fossil is, however, so unusual that its familial disposition is not readily resolved.

Four families of frogs are currently recognised from Australia: Hylidae, Leptodactylidae, Microhylidae and Ranidae. Nevertheless, the disjunct distribution of the Leiopelmatidae now confined to North America and New Zealand indicates that leiopelmatids may have formerly occurred in Australia. Hence it would be unwise to restrict comparative osteological studies to families now represented.



FIG. 1 Left ilium lacking only the proximal portion of the shaft and the distal tip of the dorsal acetabular expansion. (Photo: R. Wood.)

What makes the fossil so noteworthy is the presence of a deep lateral groove extending on the length of the ilial shaft. Modifications to the shaft certainly occur in several modern families, but such modifications inevitably involve the development of ridges or crests extending above the body of the shaft. In many ranids the crests are particularly well developed.

The moderate development and lateral disposition of the dorsal prominence is consistent with Lynch's³ observations on Australian leptodactylids, but the acute angle at which the ventral acetabular expansion subtends to the ilial shaft is contrary to Lynch's definition of 90°–110° and my own observations (90°–130°). Provisional studies, however, favour the assumption that the fossil will ultimately prove to be a leptodactylid or possibly a hyliid. Trueb⁹, the most recent contributor to revisionary studies of the anuran ilium has already summarised the criteria that support this contention, namely that primitive anurans "have a plain shaft that tends to be cylindrical in cross section". It has been argued by Tyler¹⁰ that the Microhylidae and Ranidae probably colonised Australia no earlier than the Pleistocene. Bearing in mind the duration of the isolation of Australia, it seems likely therefore that the Miocene fauna was composed solely of hyliids and leptodactylids, and possibly a perpetuated remnant of the ancestral stock from which these Australian frogs are derived.

That the Ngapakaldi Fauna is only of mid-Miocene age, relies upon the palynological evidence of W. K. Harris (personal communication), for Stirton, Tedford and Woodburne⁶ consider it late Oligocene or early Miocene. The environment occupied by the Ngapakaldi Fauna, as indicated by its content, was certainly less inimicable to anurans than the current aridity, and it follows that it is reasonable to anticipate little similarity between the Ngapakaldi anuran Fauna and the arid-adapted fauna now occurring in the extreme north of South Australia.

I thank Dr M. O. Woodburne for the opportunity to examine this material. Description awaits the completion of more detailed comparative studies.

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Erratum

Figure 3 of the article "Aftershocks caused by the Novaya Zemlya explosion on October 27, 1973" by Hans Israelson, Ragnar Slunga and Ola Dahlman (*Nature*, 247, 450; 1974) and Figure 3 of the article "Coral growth related to resuspension of bottom sediments" by Richard E. Dodge, Robert C. Aller and John Thomson (*Nature*, 247, 574; 1974) were interchanged. Figure 3 of the article by Israelson *et al.* should have been:

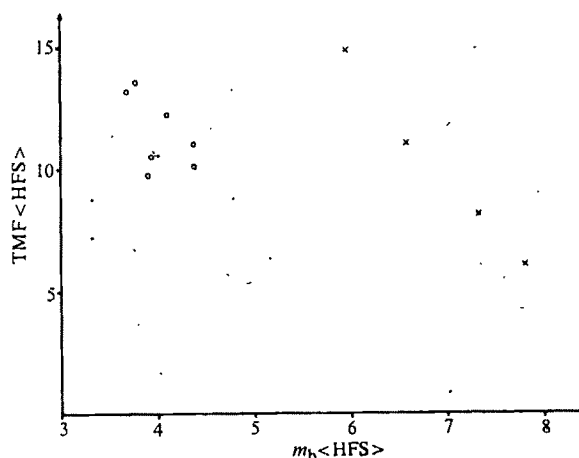


Fig. 3 Third moment of frequency, TMF, values as a function of body wave magnitude, m_b (HFS), for explosions (x) and aftershocks (o).

and Figure 3 of the article by Dodge *et al.* should have been:

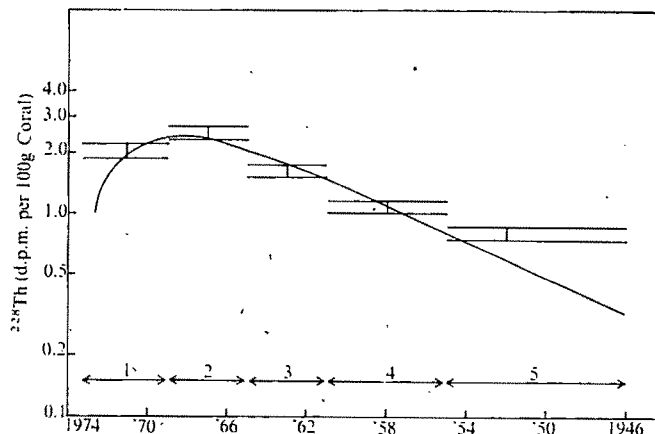


Fig. 3 ^{228}Th specific activities obtained for skeletal intervals shown in Fig. 2. The curve is the predicted ^{228}Th ingrowth for an initial ^{228}Ra value of 4.0 d.p.m. per 100 g coral.

book reviews

Chemistry to build reactors

The Chemistry of Fusion Technology. Edited by Dieter M. Gruen. Pp. xiv+394. (Proceedings of an American Chemical Society Symposium held in Boston, Mass., in April 1972.) (Plenum: New York and London, 1972.) \$22.50.

THE possibility of creating conditions in which the nuclear energy of light elements might be released in controlled nuclear fusion reactions emerged from the advances in nuclear physics in the period 1930–50. To bring these ideas to fruition, it has been necessary first to establish a major new branch of physics, high temperature plasma physics; second, this new knowledge has to be applied to potentially practical systems; and third, the technology of such systems has to be established.

With the growing progress in plasma physics and in its application to the magnetic confinement of high temperature plasmas (a turning point was the publication in *Nature* of the results of the joint Soviet–United Kingdom experiments on the Russian Tokamak magnetic confinement device in 1969) and with a growing awareness of the acute nature of the potential energy shortage, much more interest is being shown worldwide in the third area—the technology of future fusion reactors. Since very large resources have been accumulated over the years in nuclear fission technology, there is no shortage of experienced and resourceful researchers able and anxious to tackle the new technological challenge: and it is now possible for the energetic student to attend perhaps half a dozen symposia a year in which the problems of the technology of fusion reactors are scanned and discussed. Moreover, large sums of money are forecast to be available, particularly in the United States and Germany, to tackle what in 1946 George Gamow described as a problem of almost unsurpassable technical difficulty.

The present volume is a record—and a good one—of one of the most constructive of such symposia. It discusses, at a foundation level, the problems of chemical engineering which will have to be dealt with if the current physical idea proves correct: that controlled fusion reactors will be fuelled by deuterium and lithium and based on thermonuclear reactions between deuterium and tritium in an isolated high temperature

plasma. No particular system of plasma confinement or control is envisaged, so that the meat of the volume is of value whichever of the current systems advocated on physics or engineering grounds turns out to be the most practicable.

J. D. Lee of the University of California is a leading authority on the breeding of tritium in the lithium-bearing blankets, and his article (40 pages) on tritium breeding and direct energy conversion summarises authoritatively the main results of his group on neutronics of the blanket, together with short discussions of the chemical and electrochemical problems of using liquid lithium metal as a coolant in a high magnetic field. The section on direct conversion is, disappointingly, confined to a secondhand presentation of one particular system—the direct conversion of the mirror energy leakage, by R. F. Post. The review of the chemical, physical and thermal properties of lithium (70 pages, 111 references) by Cairns, Caffasso and Maroni of the Argonne National Laboratory, is a fundamental and authoritative collation of data particularly valuable because of critical assessment of the data and the extensive bibliography; it includes data on topics ranging from natural abundance, thermodynamics and physical properties to corrosion rates of selected high temperature materials in lithium. Likewise, W. R. Grimes and S. Cantor of Oak Ridge on the use of molten lithium-bearing salts as coolant and breeder (28 pages, 36 references) cover important data on one of the major alternative constituents of the blanket, and their article is based on Oak Ridge experience of the Molten Salt Reactor. E. F. Johnson of Princeton—another leading authority—deals (22 pages, 21 references) with the chemical problems of tritium extraction from the blanket, but rather too briefly and qualitatively to be of lasting value. His article does, however, give a useful summary of the Princeton reference design model reactor which provides the non-specialist reader with a concrete example of present-day reactor concepts.

D. M. Gruen provides a useful and interesting account (24 pages, 57 references) of the often-neglected chemical effect of plasma interaction with thermonuclear reactor surfaces, together with a brief review of more familiar surface topics. R. E. Stickney provides a major

and critical account (78 pages, 110 references) of the permeation of hydrogen isotopes through high temperature materials—a matter of great importance to the assessment of the spread of tritium around and through the components of envisaged reactors. Finally, the chemical content of the volume is completed by a short (16 pages, 31 references) article by G. G. Libowitz on condensed-metal hydrogen systems, and a rather superficial article on superconducting materials.

To the fusion reactor specialist, the main value of the book rests in the bringing together of today's main chemical knowledge relevant to fusion reactors, or at least fusion reactor blanket systems. To the chemist, the book indicates the main areas of chemical technological interest provoked by current fusion reactor concepts. The impression for the non-specialist is likely to be that each individual problem of chemical technology so far raised and discussed has outline solutions, but the sum total of the problems constitutes a serious technological barrier which—as in the case of fission reactors—can seem daunting in the initial stages. There is clearly a great need to improve much of the chemical data and understanding, particularly in the difficult areas of corrosion (where the effects of trace elements are not understood), permeation and radiation chemistry.

R. S. PEASE

Solid electrolytes

Physics of Electrolytes. Edited by J. Hladik. (Transport Processes in Solid Electrolytes, and In Electrodes vol. 1. Pp. xiii+516. (Academic: New York and London, August 1972.) £11.00; \$34.

THIS series of review articles was presumably intended to provide the background knowledge required for investigating the properties of ionically conducting solids, a field which is currently attracting considerable interest. The topics covered in this volume are grouped into two sections; "Solid Electrolytes and Electrodes" and "Transport Processes".

The first section contains a rather heterogeneous collection of articles. In the first two chapters, Hladik discusses electronic theories of the solid state, interatomic distances, the cohesive energy in ionic crystals and also gives a gen-

eral account of solid electrolytes. Unfortunately, the relevance of many of these topics to the study of solid electrolytes is not made clear. The discussion of interatomic distances is rather inconclusive and that on cohesive energy suffers from a lack of references to the tabulated data; this is particularly irritating when the data in two tables (X and XII) do not agree.

Fong has contributed an account of the statistical mechanical treatment of interactions between impurity atoms and lattice defects in ionic crystals, and the experimental results available to test this theory. In the final chapter of this section, Amsel describes the range of applicability of, and some of the experimental results obtained with nuclear microanalysis. This chapter makes extremely interesting reading but deals mainly with film formation on metal surfaces.

The section on transport processes is much more homogeneous. Friauf gives a sound account of the basic theory of conduction and diffusion in solids and describes the methods available for determining the mechanism of these processes. Diffusion processes are also clearly discussed, though in more detail, by B  ni  re but there is considerable overlap between these two chapters; some of the tables and figures and much of the theory being common to both. B  ni  re has also contributed a good but incomplete account of transference number measurements in ionic crystals. The measurement of the transference number of the electron by EMF measurements or by the Wagner technique is unfortunately not described. Ionic conductivity in solids is reviewed by Kvist but only a cursory account of the experimental methods and the conditions which must be satisfied in making conductivity measurements is provided.

The remaining chapters by Hartman, on ionic conductivity in whiskers, Hughes and Isard, on ionic transport in glasses, and Riande, on transport phenomena in ion-exchange resins, are very competent reviews.

Overall, this book suffers from inadequate editing to remove the unnecessary overlap between some chapters and to provide the links between the separate contributions. The proof reading, especially of the chapters by Hladik, must have been careless and should have ensured that the tables and diagrams in the chapter by Kvist appeared nearer to the relevant sections of the text.

In spite of the limitations described above, this collection of articles and especially those on transport phenomena, should be of value to those involved in this area of research.

T. DICKINSON

Copernicana

The Scientific World of Copernicus: On the Occasion of the 500th Anniversary of His Birth, 1473-1973. Edited by B. Bienkowska. Pp. xii+142. (Reidel: Dordrecht and Boston, 1973.) Dfl. 50.

1973, half a millennium since the birth of Nicholas Copernicus the founder of modern astronomy, has produced an unprecedented outpouring of Copernicana. To the present example twelve authors contributed essays, three previously published in Polish and one in French.

Discrepancies between statements by different contributors have been overlooked by the editor. Thus Copernicus is said to have spent "five years as [a] student in Cracow" (page 1). Yet "Copernicus enrolled at the University of Cracow in 1491. For the four years he remained at the University he devoted himself to the study of the liberal arts without, however, winning an academic degree" (page 15). Was Copernicus at Cracow four years or five? The university records do not reveal when he left. At Cracow the bachelor's degree was normally obtained in four years. Surely the founder of modern astronomy was no dullard. Since Copernicus received no degree from Cracow, and did not need the baccalaureate for the career which he contemplated, presumably he stayed less than four full academic years in Cracow.

Readers are assured that the thesis of Copernicus' *Revolutions* "was accepted . . . without demur by Pope Paul III, to whom Copernicus' book was dedicated" (page xi). For this familiar assertion about the reigning pope, nobody has ever adduced any documentary support. Evidence to the contrary was published in *Rivista critica di storia della filosofia* (26, 84-85; 1971). Shortly after Copernicus' *Revolutions* was printed in 1543, initiating modern astronomy and therewith modern science, preparations to condemn that magnificent work were begun by Pope Paul III's personal theological advisor, Bartolomeo Spina; the Master of the Sacred and Apostolic Palace. But before Spina could act, he fell ill and died. Thereupon Spina's close friend Giovanni Maria Tolosani resumed the theological anti-Copernican campaign in an appendix to his treatise *On the Truth of the Holy Scripture*. Herein Tolosani declared that Copernicus "contradicts some Scriptural principles, not without danger of infidelity both to himself and to the readers of his book". An annotation in Tolosani's manuscript discloses that his attack on Copernicus "for the purpose of safeguarding the truth to the common advantage of Holy Church" was later consulted by the preacher of

the first public sermon against that great Copernican, Galileo.

The (lost) reconciliation of Copernicanism with the Bible was written by Copernicus' disciple Rheticus, not by Bishop Giese (page 27). The "first publisher" of Copernicus' *Revolutions* was Petreius, not Osiander (page 140).

EDWARD ROSEN

Chromosome more or less

Cytogenetics of Aneuploids. By Gurdev S. Khush. Pp. xii+301. (Academic; New York and London, December 1973) \$17.50: £8.40.

THIS book is a survey of what has been achieved in isolating, identifying and using aneuploids, that is, individuals whose chromosome number differs from an exact multiple of the basic number. Most of the book is concerned with aneuploids in plants, with a very short chapter in which the author briefly describes examples of aneuploids in animals, especially man.

After outlining concisely the history of the discovery and production of the various aneuploids, and the terminology used for the different types, the author gives a very useful glossary of the current terms, and an explanation of the chromosomal formulae used to indicate the different types of aneuploids.

The book is then divided into two main parts, the first dealing with trisomics, with some mention of tetrasomics, and the second dealing mainly with monosomics but also mentioning nullisomics. As monosomics and trisomics have seldom been developed in the same species, this does not lead to too much duplication, and the two sections are linked by cross references where necessary. Each part covers the sources of the relevant aneuploids, detailing how the various types have been obtained in different species; some of the difficulties encountered in producing the aneuploids; the cytological behaviour associated with the aneuploid condition; and the breeding behaviour of the aneuploids. There are chapters on the uses of aneuploids, which include chromosome mapping, locating markers on chromosomes, locating the centromere and orientating the linkage map on the chromosome, as well as the production of substitution and addition lines.

The book brings together data widely scattered in the biological literature, and so will be a valuable reference work for those wishing to develop or use an aneuploid series, as well as being a useful source of information for anyone requiring a basic knowledge of the subject. It will therefore be of use to honours undergraduates as well as practising cytogeneticists and plant breeders.

J. P. MOSS

Full attention

Attention and Effort. By Daniel Kahneman. Pp. x+246. (Series in Experimental Psychology.) (Prentice-Hall: Englewood Cliffs, N.J., July 1973.) \$8.95.

BETWEEN the wars the topic of attention was neglected by psychologists, in their enthusiasm for predicting each response as a function of the immediately previous stimulus. The difficulty was, however, that the effects of a stimulus depend upon the state in which a man is when he receives it. Sometimes he reacts to it, will say verbally that he has noticed it, will remember it later; and sometimes none of these things will happen. The processes which can be variously and roughly described as gating, filtering, or selecting among inputs to the system have therefore become a live area of research: and books on attention are now almost too numerous. They range from the massive, detailed, and unreadable to the popular, chatty and superficial. The competition for another is tough.

It is a pleasure therefore to be able to declare this book the best one on the subject thus far. It is short, clear, and extremely up to date: not merely are there references to publications in 1973, but also exciting accounts of new experiments by the author and his students in Jerusalem, which will be carefully read by advanced workers in the field. At the same time, the coverage of the literature is wider than in most of the competitors, and the author understands sympathetically what is being argued by other investigators. Thus for once this is a book which can be recommended, not only to the advanced workers already mentioned, but for teaching purposes, and even conceivably for non-psychologists who wish to find out the latest state of the art in this area.

For those who are already expert, one can indicate the position the book adopts by saying that it takes broadly the position of Treisman, but with much greater emphasis and experimental detail on Neisser's pre-attentive processes, and on recursive influences from processes at one stage of recognition upon others both earlier and later in the system. The findings of the past two or three years make it possible to do this convincingly and briefly, and Fig. 5-1 is perhaps the first diagrammatic representation of attention which improves upon that of Treisman in 1960.

Criticisms? The book is weaker when it turns to motor performance rather than perception, which is reasonable because the state of knowledge is less satisfactory: and there are perfectly adequate references to Posner, to Keele,

to Brown and to Trumbo and Noble for those who wish to go further. Those of us who work on the effects of noise and of other stresses will be discontented by the way in which effort, arousal, the allocation of resources and the process which determines the size of the pupil are all treated almost interchangeably. Perhaps one can most fairly put it this way: there are real differences, which experimental evidence compels us to draw, between easy and involuntary attention such as that with which we follow the graceful prose of this author; and the determined concentration, sustained by a hope of remote goals, with which we mobilise all our resources to follow the confused thinking of some others. These differences are not yet understood, but for what we know now, Kahneman is the best guide.

DONALD E. BROADBENT

Nematode interactions

Nematode Ecology and Plant Disease. By H. R. Wallace. Pp. ix+228. (Arnold: London, December 1973.) £6.50.

THIS is a readable and interesting account of nematode ecology and the role of nematodes in plant diseases. Early chapters discuss nematode injury to plants, plant growth in relation to numbers of nematodes and plant responses to nematode invasion and feeding. The second half of the book deals with distribution, abundance and adaptations of plant-parasitic nematodes in the environment and with their involvement in specific diseases. The final chapter suggests how the role of nematodes in plant diseases should be investigated.

Unlike Dr Wallace's earlier book (*Biology of plant-parasitic nematodes*; 1963) this is no textbook; for it is far from comprehensive and the references are nearly all drawn from the last decade. The text is often speculative, not a bad thing, but it is also at times superficial, and with some conclusions that are debatable. The book is best where, as in parts of the chapters on nematode ecology, it deals concisely with its subject. Too much of the text, in my opinion, is taken up with fulsome descriptions, definitions and debates on abstractions such as 'health', 'disease', 'homeostasis', and ecological jargon and with the views of medical and plant pathologists.

Too little attention is devoted to well documented evidence of the relationship of nematodes with plant diseases. For example, much is known of nematode-fungus interactions in plant disease complexes and of nematodes as vectors of plant viruses, both of which receive scant attention. The inferences drawn about the work on Docking disorder are incorrect. Nor does Wallace indicate

how much can be learnt from careful study of plant symptoms and from inoculation experiments with suspected pathogens.

Instead, he stresses the use of multifactorial analyses of all the environmental variables that can be thought of and measured, to indicate which factor or combination of factors is most likely responsible for the particular disease. To my mind this is putting cart before horse. Not all diseases involving nematodes are as complex as Dr Wallace implies or we would be without commercially successful cultivars bred for resistance to specific nematodes. Despite such criticisms I commend this book to all thoughtful nematologists, plant pathologists and ecologists.

A. G. WHITEHEAD

Physicists talking

Fundamental Interactions in Physics: 1973 Conference at the Centre for Mathematical Physics in the University of Miami. Edited by Arnold Perlmutter. Pp. ix+399. (Studies in Natural Sciences vol. 2.) (Plenum: London and New York, 1973.) \$29.

A CONFERENCE on so wide a subject as this one is in danger of losing coherence and producing a set of papers from so great a range of disciplines as to require from any one scientist an enormous effort of comprehension, and yet to include contributions so speculative as to make such effort unworthwhile. This familiar danger is avoided by the present volume, largely because of the elegance and informality of the presentation of the papers which seem to have been presented as talks and recorded verbatim. Here, perhaps, is a solution to the problem of scientific style on which John Maddox commented in a recent BBC radio broadcast: "... when a scientist sits down in front of his IBM typewriter. . . ."

There are three sections in the book. The first deals with particle physics and is largely theoretical though it includes an informal description of work at the National Accelerator Laboratory (Batavia, Illinois) and a paper by Hofstadter on applications of absorption detectors. The theoretical papers range from a quasi-classical discussion by P. A. M. Dirac of symmetry breaking, in a new variation of Weyl's theory of the electromagnetic field, to a terse and wholly algebraic "Approach to Hadron Physics . . ." by Oneda and Matsuda. Several of these papers contain much that is new, and some of them contain fertile ideas rather than completed theories. For example, Bopp and Lutzenberger in a short contribution throw out a simple model of quantised retarded forces in which the electron and the muon appear as eigensolutions sub-



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ject to the correctness of guesses about the bare masses and angular momenta of the particle in their model. But at least three of the papers (by Kur-sunoglu, Salam and Blokhinstev) have a more didactic purpose and will be of great use to graduate students of particle physics.

The papers in the second section on atomic and molecular physics, one by Massey, one by Spruch and one by Herzenberg, are nearer to reviews than those in the first, but there are also two panel discussions. Three more speculative papers on X-ray astronomy, helium and biology make up the third section.

Despite the relatively high speed of the book's production the presentation is excellent and free of misprints. Thus a scattered but uniformly high powered, original but well written collection of papers make up a book which particle physicists will find stimulating and informative. I hope that university libraries (to whom the high price presumably restricts the book's sale) will make it available to them.

PHILIP ROE

Thinking protozoa

The Evolutionary Foundations of Psychology: a Unified Theory. By Felix E. Goodson. Pp. xi+228. (Holt, Rinehart and Winston: New York and London, 1973) n.p.

Dr Goodson sets himself the Kantian goal (the parallel is mine) of effecting a transcendental deduction of the psychological factors necessarily involved if the evolution of complex organisms is to be possible. In other words, he aims for an integrated view of psychology derived by way of abstract arguments from a broadly evolutionary base.

Unfortunately, although he has some interesting things to say about the psychological necessities inherent in the earliest forms of life, his concepts are not sufficiently structured or precise to add much to one's understanding of mammalian and human psychology. To be sure, his stress on adaptive function for the sake of the organism as a whole is preferable to an excessively narrow conception of psychology that ignores man's evolutionary context. And he relates a wide variety of findings in empirical psychology to this overall perspective. But his remarks are too vague to be helpful, and leave most interesting substantive questions unanswered—or, worse, unasked.

For example, he identifies the paramount task of psychology as "an evaluation of apperception that focusses on the variables that affect the rapid shifting from one component to the next", where by "apperception" he means the process (often called "attention") by which inputs from the senses or the

memory come briefly into focus in the conscious mind (page 68). Whether or not accounting for the detailed course of the stream of consciousness is 'the' paramount task of psychology, it certainly is an important psychological problem. But what does Dr Goodson have to say on the subject?

"Thinking", he says, "is nothing more than fluctuation of apperception among available encodes and as such is a completely automatic and lawful process" (page 135). Readers of *Nature*, who are not prone to think of thinking as a random activity, can hardly demur with this—but what one wants is some indication of the nature of the laws involved. Precise specification, of course, is not to be looked for in a deliberately general and abstract discussion. But one might reasonably hope for a more powerful concept than "equilibration" (which is involved in Goodson's most basic "evolutionary postulates of process") an indication of the psychological complexity of thought. Thus the characterisation of "thinking" quoted above continues: "Apperception is always the most equilibratory response possible to the myriad components dynamically interacting within the integration matrix, and when the components brought into sequential focus consist of encodes, thought is taking place".

If a generic concept of equilibration is to be applied equally to the specifics of chemical diffusion in protozoa and flights of fancy in human beings, more needs to be said about the differential aspects of the phenomena so confidently classified together. Even the social psychological theories of "cognitive consistency" and "cognitive balance", vague and unsatisfactory as they may be, make a better attempt than Goodson to state what constitutes "equilibration" in differing types of thought. Equally, Freud offered more substantive suggestions about the structure of the "integration matrix" and the varieties of "psychic disequilibrium" than can be found in this book. It is ironic that Goodson castigates psychoanalytic theory for "its reliance on vague terminology" (page 204).

I should perhaps admit my own conviction that the complexities of mental structure can only be satisfactorily approached by way of the concepts currently developing within artificial intelligence. My dissatisfaction with this book doubtless reflects the measure of its distance from this theoretical approach. But I think it unlikely that psychologists favouring other paradigms would find it a rich source of insights. Even Kant tried to show in detail how specific examples of human thinking (such as the metaphysical antinomies) were generated by the abstract forms of thought postulated by his transcendental deductions. MARGARET A. BODEN

science on radio

Bush House bonanza

John Gribbin and Eleanor Lawrence

One of the strangest economies proposed in the latest round of British belt-tightening is to cut back drastically on the funds available to the external services of the BBC, which operate from Bush House, London. These services have already suffered a small decline in the amount of cash available to them—which represents a significant decrease in real terms, after inflation has done its worst. Yet the BBC continues to be held in the highest esteem by listeners around the world, who find its services informative and, relatively, free from propaganda. Of course, even the BBC is not completely unbiased: its science, industry and agriculture unit, for example, makes no bones about the fact that although it hopes to report fundamental developments in pure science wherever they are made, technological applications with commercial applications are generally only 'promoted' if they are British. But that understandable bias is hardly in the same league as the political propaganda of the other giants of world broadcasting.

Compared with the domestic services of the BBC (and, indeed, the internal broadcasting services of other countries) Bush House provides a veritable scientific bonanza. Out of 168 hours of broadcasting in English each week, there are two half-hour programmes devoted entirely to science (each of them repeated twice), a nature notebook and a programme for farmers. With more or less regular scientific features (12 to 20 a year, each running for 30 minutes) and the tit-bits of science found in news and current affairs programmes, the total is something like 5 percent of the weekly output.

This is achieved with a very restricted budget, roughly half of the amount available for comparable domestic programmes (such as the late, lamented "Kaleidoscope"); if the funds of the science unit are cut back at all there seems no way in which economies could be made except by reducing the number of programmes. It may not happen, and it certainly should not happen, to judge from the quality, as well as the quantity, of these programmes.

Of the two main science unit programmes, "Science in Action" aims "to inform and sometimes amuse" the non-

specialist listener. We were a little surprised by the high scientific standard of this programme—but of course it is broadcast in English, and therefore its audience overseas must be better educated and more well-informed than the average population. Audience response and a recent survey indicate that medical items are most popular, with communications and astronomy following some way behind. Questions asked by listeners indicate how successfully the programme communicates with its intended audience: would two children brought up together in isolation from birth learn to communicate and what are hypertension and vertigo?

At a high level, "Discovery" aims to be by scientists and for scientists, and beats anything else we have heard on domestic radio or elsewhere hollow. In the half-hour programme, two or occasionally three scientists are interviewed about their own work and given free reign. The result is something like a radio version of the "News and Views" section of *Nature*.

In one programme we heard, Dr V. Marks from the University of Surrey talked about gut hormones and insulin control, and Dr Jeffrey Manning from the Rutherford Laboratory explained their current accelerator research programme. This did not duck what seemed to us the contentious question—especially to an audience in the developing countries—of whether an accelerator costing £15 million is justifiable. But it seems that there is, in fact, a more general recognition of the value of fundamental research in the countries which can least afford it themselves.

This raises another interesting point: the lively postbag received by the science unit includes contributions from the poorest countries, where the cost of posting a letter to Britain can be as much as 50 percent of a week's earnings, yet many listeners feel it worth the cost of responding in this way. One correspondent, an Indian doctor, said how much he valued the programmes as his only effective means of keeping up to date, since the cost of "the literature" is quite prohibitive for him.

Part of this listener interest must stem from the immediacy of the programmes. Special features, such as one discussing the causes and implications of the present droughts in sub-Saharan Africa, can be put together within 72

hours, and in a "Science in Action" programme broadcast in the week ending April 6 Dr Simon Mitton, of the University of Cambridge, could be heard giving details of the Mariner-10 observations of Mercury which were certainly new to us. This immediacy is aided, of course, by the happy relationship between Bush House and active scientists, who are, it seems, often pleasantly surprised at the difference in approach between the external services and the domestic TV services.

As well as the programmes broadcast from London, the BBC also provides a service for radio stations around the world in the form of taped programmes. And this really is a service—a weekly fifteen minute tape programme, for use intact or to be cut up and inserted into other programmes, costs only £1.50 plus postage, and there is no obligation for the user even to credit the BBC when an item is used. One of these tapes, "Science Magazine", has a link with *Nature* in the form of a weekly contribution from one or more members of the editorial staff: this programme is a mixture of short items and a long interview, almost a combination of "Science in Action" and "Discovery". It has not always come off as a programme in itself, although at the giveaway price excerpts find outlets around the world, not just in developing countries but also on American public and campus radio. We have, however, heard a pilot of a 'new look' "Science Magazine", with more punch and a lively signature tune: this format seems more likely to provide a good listen and makes more sense for users who wish to broadcast the programme intact.

All in all, the science output from Bush House is difficult to fault. The fundamental reason for this seems to us to be that the producers themselves and most of the presenters all have scientific training, although the audience is not entirely science oriented, as correspondence from insomniacs and long distance lorry drivers in Britain indicates. Far from reducing this valuable output, the powers that be should be encouraged to promote it as a valuable string to the BBC's bow, in the best traditions of Reith. Perhaps they could even take note of the most common plea from those insomniacs and list the programmes, in as small a print as they like, in the *Radio Times*.

instrument review

Surround sound

John Wilson

IN an attempt to capture both the natural direction and reverberation of sound in a live performance, Professor Peter Fellgett of the University of Reading and Michael Gerzon, an Oxford mathematician, are developing a new 'ambisonic' hifi system in association with IMF, a British loudspeaker manufacturer. The National Research Development Corporation holds the patents for the system and is backing the project financially.

The new technique improves on present quadrasonic systems because of its ability to present natural sound images between the front and rear pairs of speakers, and to reproduce sounds which seem to arise either between the listener and a loudspeaker or beyond it. Indeed Mr Gerzon believes that: "Quadrasonics, as at present widely conceived, is a dead end."

Unlike the conventional quadrasonic approach, where a separate channel is so often deemed necessary for each loudspeaker, the new ambisonic system uses information from a multidirectional microphone array encoded on just two channels. This should lead to the complexities and sophistication of surround sound techniques being relegated to the recording studio and not to the living room. Indeed, apart from two extra loudspeakers, suitably in phase, only a decoder will be necessary to convert an ordinary stereo system.

This new approach should not be confused with the so-called 'matrix'

systems where information from conventional microphones is artificially blended to achieve an approximation of surround sound. With ambisonics, sound from every direction reaching the tetrahedral microphone array is treated equally until the decoding operation.

Michael Gerzon, who Professor Fellgett feels should take most of the credit, points out that an ambisonic system is not limited to four loudspeakers, although this is the number which is most attractive commercially. Since the microphones also pick up the sounds coming from above and below them, these too may be reproduced if six or more loudspeakers are used with the appropriate decoder. Three is the optimum number of channels for four speakers (and therefore fm radio transmission), five channels for six loudspeakers, six for seven and so on. The position of the loudspeakers must be very carefully worked out because they are not in the usual planar arrangement.

Nature hopes to publish an article describing the mathematics behind the new system in the fairly near future; but how does it sound in practice? The new system was demonstrated at the recent Sonex 74 exhibition in the Post House Hotel near London's Heathrow airport. Four expensive monitor speakers were supplied for the occasion by IMF but the signals themselves came from an ordinary Philips cassette.

Just before the demonstration began, a director of IMF, Mr John Wright, said a few words of warning about the acoustics in the demonstration room. He should have said more because the organisers could hardly have picked a

worse spot. One wall of polished mahogany bounced the sound like a mirror through an acoustically transparent partition opposite and even the other, more normal, wall was faced by a large window with heavy, absorbent curtains. So the demonstration was rather disappointing, particularly regarding the claim that the nearer a listener is to particular speaker, the less sound he is conscious of hearing from it. This simply could not be true in a room where the sound was escaping next door through one wall and being smothered in the curtains of another.

In spite of this one or two of the selections on the tape gave some idea of what an ambisonic system is capable. In particular, a fine piece of organ music produced the strong impression of being in an echoing church. Considering the real surroundings, this was no mean feat and Professor Fellgett said that he hopes to arrange another demonstration in a more suitable location.

The technique is still very much in the experimental stage, with new directional microphones being developed, for example, and the inventors emphasise that they have no plans yet to market it themselves. Rather, they wish to attract the attention of recording studios and manufacturers with a view to licensing their invention—much in the same way as Dolby has licensed his method for 'stretching out' the hiss from tape recordings. In this respect Professor Fellgett says that IMF is acting like "the good farmer" by seeking to develop the system for the benefit of all, although it naturally has a commercial interest in being in the forefront of a new technique.

matters arising

The information problem

SIR,—K. L. and M. L. Blaxter, in their article *The individual and the information problem* (*Nature*, 246, 335) make some incidental remarks which cannot pass unchallenged. They say, for example, that the "so-called information explosion . . . apparently causes consternation to librarians, archivists and information scientists". No evidence is quoted

in support of this suggestion, perhaps because it would be difficult to come by. Whatever consternation exists is, in fact, more likely to be found among the ranks of the users than of those of us professionally engaged in dealing with the problem. We are far too busy actually harnessing the forces of the information explosion to have time to strike attitudes of consternation.

Bryan¹ summed up the viewpoint of

many librarians when he argued that the information problem is essentially a users problem, and not primarily a technical problem. The remark is also unfortunate in that it seems to imply an inactivity in the face of crisis; a glance at, for example, the current issue of *Library and Information Science Abstracts* may help to dispel this misconception. Most of the Blaxters' findings are already well known to librarians, and we need no convincing

as to the desirability of such things as the application of objective criteria to the purchase of periodicals. At least one university library, Surrey, is now basing its periodicals purchasing policy on studies of use², and the possible use of such aids as the Institute of Scientific Information's *Journal Citation Reports* is being actively examined in other libraries. Secondary journal citation is another approach being studied³.

It is also suggested that for a scientist to admit that he could not keep abreast of his own speciality would imply an incompetence. There is a confusion here between two separate and distinct areas of competence; competence as a scientist in a particular field of science, and competence as an information searcher. These two abilities are different, and the first does not necessarily imply the second. Many writers have commented on the difficulties encountered by scientists in using the literature⁴⁻⁸, and some scientists are prepared to admit their limitations in this field⁹. If past work is to be fully utilised, and duplication avoided, it is essential for all scientists to be aware of their own personal limitations, and to seek whatever assistance they require from professional information workers.

As long as there remain scientists who regard it as somehow *infra dig* to ask for advice or help on information problems, then some at least of these problems will be of their own making.

Yours faithfully,
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¹ Bryan, H., *Aust. Lib. J.*, 17, 389 (1968).

² *Annual Report, 1972-1973* (University of Surrey Library, 1973).

³ Windsor, D. A., *Spec. Libr.*, 64, 446 (1973).

⁴ Urquhart, D. J., in *University Grants Committee, Report of the Committee on Libraries*, 280 (HMSO, London, 1967).

⁵ Urquhart, D. J., *Aslib Proc.*, 18, 351 (1966).

⁶ Wood, D. N., and Barr, K. P., *J. Doc.*, 22, 22 (1966).

⁷ Bottle, R. T., in *Progress in Library Science 1967* (edit. by Collison, R. L.), 109 (Butterworths, London, 1967).

⁸ Line, M. B., *J. Libr.*, 1, 211 (1969).

⁹ Schur, H., and Saunders, W. L., *Education and Training for Scientific and Technological Library and Information Work*, 37 (HMSO, London, 1968).

The individual and the information problem

SIR,—Blaxter and Blaxter raise a number of interesting points in their recent paper (*Nature*, 246, 335; 1973). They are careful to emphasise that their results and conclusions obtain from the views and habits of full-time research

scientists working in three biological research institutes, and that these may well differ from those of university lecturing staff, with educational commitments in addition to those of research. I should like to comment here on just one conclusion from the Aberdeen survey, however, and suggest that it may indeed be more generally applicable.

The Blaxters were able to estimate a figure for the total number of journals to be taken by a research institute's library to give 90% satisfaction to 90% of its users, a figure which was 25 times the number of 'fields' covered by the institute. The concept of a 'field' was defined by the publishing patterns of the individual scientist and the whole institute. For instance, for the institute cited, the number of non-overlapping fields was calculated to be five, so that the theoretically optimal number of journals to be taken by the library was only 125.

In 1971 a survey was conducted in Edinburgh by interviewing a representative proportion of the staff of the medical school with the main purpose of obtaining an estimate of the journals that our central medical library ought to take to give reasonable satisfaction to its readership. Our survey was designed rather differently from the Aberdeen one and full details of its methodology and general results have been published¹. From the results, we were able to obtain a total figure of approximately 580 unique primary and review journals covering all the major subject areas of biomedicine, except for social medicine, public health and dentistry, which were outside the scope of our survey. I must admit that we were pleasantly surprised and relieved that the figure was not higher.

For the library of a research institute, a holding of 580 carefully chosen journals is theoretically optimal for 23 non-overlapping fields with 25 journals per field, by the Blaxters' method of analysis. In the Edinburgh medical school survey, the 580 unique journals were in fact derived almost entirely from 47 subject areas as defined by the Index Medicus List of Journals. This corresponds to an average of 12 to 13 unique journals per subject area. Many individuals in our survey had an interest in the journals of more than one subject area, and between the various medical school departments there was evidence for considerable overlap of interest. It would therefore seem not unreasonable if a 'field' in the Blaxters' sense approximates roughly in size to two average subject areas in the Index Medicus sense.

Approximate techniques inevitably have to be used to estimate the optimal journal holding for a library serving a readership of any complexity. The important point is that, though based on

different methodologies, both the Aberdeen and Edinburgh surveys produced the same conclusion: in spite of the information explosion, the optimal journal holding of a research institute or medical school library appears not to be so enormous after all, given, of course, adequate back-up facilities by a national loan service. In fairness to Edinburgh's medical school and library staff, I should add that financial restrictions have prevented our central medical library from as yet achieving the estimated optimal number of journals.

Yours faithfully,

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¹ Whittle, E. D., *Br. J. med. Educ.*, 6, 306, (1972).

Cognitive Subjective

GREGORY¹ should have acknowledged sources in references to his experiments and discussion of what he calls "cognitive contours" or, as others would have it, "subjective contours". ("Subjective contours" represents a neutral description, whereas "cognitive contours" suggests the role of an intellectual process.) He acknowledges Kanizsa as the source for his Fig. 1, but does not do so for his Fig. 2c, a figure also to be found in Kanizsa (Fig. 13)². Gregory reports, in regard to one of his experiments, that the subjective contours of the Kanizsa figure can be obtained stereoscopically by proper presentation of parts to each eye. I had already reported the positive results of this experiment with a slightly modified Kanizsa figure, also showing diagrammatically the selection of parts for presentation in a stereoscope (page 296, page 405 n. 14; ref. 3). In another experiment, he points out that the sides of the subjective triangle appear curved when the edges of the sectors are not collinear, an effect which he describes as "new". I had, however, also pointed this out: "The Kanizsa diagram itself can be modified so that the subjective contours are curved. The alignment of the edges of two sectors constitute a condition for straightness of subjective contours . . . When the sectors are redrawn so that the edges are not aligned, a triangular shape with curved contours is seen" (page 404; ref. 3). Furthermore, I had noted the presence of subjective contours in the after-image of an incomplete figure (page 406 n. 22; ref. 3), as had also Gregory.

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Received October 27, 1972.

- ¹ Gregory, R. L., *Nature*, 238, 51 (1972).
- ² Kanizsa, G., *Rivista di Psicologia*, 49, 7 (1955).
- ³ Pastore, N., *Selective History of Theories of Visual Perception: 1650-1960* (Oxford University Press, New York, 1971).

Nature regrets delay in publishing this manuscript. A part of this delay can be attributed to the transition from one Editor to another.

Announcements

Appointments

A. L. Armitage, Vice Chancellor of the University of Manchester, has been elected Chairman of the Committee of Vice Chancellors of the United Kingdom.

The following have been elected Fellows of the Royal Society: W. F. Bodmer; W. R. Boon; K. Burton; H. J. F. Cairns; R. Y. Calne; D. R. Curtis; J. F. Davidson; J. D. Dunitz; P. V. Edman; J. D. Eshelby; J. Halpern; S. W. Hawking; V. Heine; R. A. Hinde; A. E. Litherland; J. E. Lovelock; D. H. Matthews; R. E. F. Matthews; P. D. Mitchell; S. V. Perry; N. J. Petch; J. R. Philip; J. C. Polkinghorne; C. W. Rees; J. Rishbeth; R. V. Short; J. T. Stuart; R. H. S. Thompson; R. J. Vane; F. J. Vine; S. E. Words; and P. H. J. Young. D. H. Wiffen has been appointed Dean of the Faculty of Science of the University of Newcastle upon Tyne. Sir Eric Roll has been elected as Chancellor of the University of Southampton.

Awards

The Royal Geographical Society's Medals and Awards have been given to the following: C. Bonnington (Founder's Medal); G. de Q. Robin (Patron's Medal); C. A. Fisher (Victoria Medal); H. H. Lamb (Murchison Award); Captain D. W. Haslam (Back Award); M. A. J. Williams (Cuthbert Peek Award); A. R. H. Baker (Gill Memorial); Colonel J. M. Adam and I. Douglas-Hamilton (Ness Awards); and D. Bartlett and J. Bartlett (Cherry Kearton Medal and Award).

The Council of the Royal Society of Victoria has awarded Research Medals for 1973 to D. Metcalf and J. H. Willis.

W. T. Elwell of Imperial Metal Industries Ltd has been awarded the Gold Medal of the Society for Analytical Chemistry.

Missellaneous

The International Health Foundation (1 place du Port, 1204, Geneva) has announced that the theme chosen for its award scheme this year is **Oral Contraception—a 1974 critical assessment**. Applications are invited by the Council of the Royal Society (6 Carlton House Terrace, London SW1Y 5AG) for two Royal Society J. Sainsbury Research Fellowships.

Erratum

In the obituary of C. A. Coulson (*Nature*, 248, 367 (1974), who died on January 7, 1974, it was stated that he entered Trinity College, Cambridge from his local Grammar School. In fact he was educated at Clifton College, Bristol.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

- The Mental Health Trust and Research Fund. Annual Report and Accounts, 1973. Pp. 28. (London: The Mental Health Trust and Research Fund, 8 Wimpole Street, W1, 1973.) [1912]
- UKAEA—Research Group. AERE-R 7540: Radioactive Fallout in Air and Rain—Results to the middle of 1973. By R. S. Cambray, Miss E. M. R. Fisher, A. Parker and D. H. Peirson. Pp. iii+38. (Harwell, Berkshire: Environmental and Medical Sciences Division, Atomic Energy Research Establishment. Available from HMSO.) £1 net. [1912]
- International Computers (Holdings), Limited. Annual Report and Accounts, 1973. Pp. 24. (London: International Computers (Holdings), Ltd., 1973.) [1912]
- Provisional Geochemical Atlas of Northern Ireland. (Technical Communication No. 60). Pp. 30. (London: Applied Geochemistry Research Group, Imperial College of Science and Technology, 1973.) [2012]
- Roraima: Report of the 1971 British Expedition to Mount Roraima in Guyana, South America. By Adrian Warren. Pp. v+152. (Cobham, Surrey: Adrian Warren, Whiteoaks, Harebell Hill, 1973.) [2812]

Other Countries

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Twenty-one years of the double helix

WE celebrate in this issue the twenty-first anniversary of the appearance of J. D. Watson and F. H. C. Crick's short paper *A Structure for Deoxyribose Nucleic Acid* in *Nature*. A few will be offended by the equating of the twenty-first birthday with the process of coming of age when in the past few years the age of maturity has dropped to eighteen in many countries. We can do little for them. Rather more, with good cause, will say that there were earlier milestones than 1953 which marked the turning point in the fortunes of molecular biology. 1943 was a key year, with Avery's work on pneumococcus DNA at the Rockefeller Institute and Beadle and Tatum's on *Neurospora* at Stanford. As Macfarlane Burnet put it in *Genes, Dreams and Realities*, genetics was by these experiments directly implicated in biochemistry through micro-organisms. Others will also have their favourite years during which a quickening occurred, but few would carp at the Watson-Crick announcement being given pride of place as a starting point of something absolutely new and some (though perhaps not many physical scientists—either through ignorance or conviction) might go along with Medawar in calling it "the greatest achievement of science in the twentieth century".

We have tried to give a varied view of the history, present scene and prospects in molecular biology, from Chargaff's iconoclastic essay to Gurdon's report on the exciting developments in introducing macromolecules into living cells. One thing has become clear. A central contribution of the magnitude of Watson and Crick's stirs up the culture of science to a quite remarkable degree, and it is a change in the cultural climate, perhaps more even than the actual discovery itself, which breathes new life into science. Suddenly there are all sorts of new questions of history, philosophy, method and attitude to occupy the minds of the best scientists, along with the starker scientific questions and the less edifying ones of funding, organisation and priorities. Cultural revolutions in science are rare and immensely stimulating to those touched by them.

From the historical point of view an interesting aspect is the way in which the structure came to be accepted. As Brenner puts it; there was initially only a small band of believers and a positive effort had to be made to convince the biological community both of its correctness and profound relevance. This conforms to a classical view of a seminal idea's acceptance—first accepted by the few, later sweeping all before it. Contrast it with a more recent revolution in the earth sciences. There a whole army was waiting to pick up the central dogma of plate tectonics and make it their own. Was Watson-Crick the last great scientific idea that needed missionaries? It

seems unlikely. Although the number of scientists waiting for things to drop into their lap is greater than ever before, there is no sign that the community is any more sensitive to revolutionary ideas, however plainly presented.

The philosophical debates stirred up by molecular biology almost seem like a re-run of some of the issues generated by the explosive advances in physics early in this century. In the 1920s Eddington was talking in *The Nature of the Physical World* of his two tables; one a commonplace, substantial table—a "thing"; the other a scientific table, pervaded by fields of force and electric particles—an "influence". He clearly distinguishes between a "familiar world" and a "scientific world revealed by physics". The present debate in biology between reductionists and holists seems to resurrect all these old distinctions. Is a biological system more than the sum of the properties of its parts? For the philosopher, molecular biology has posed some fine questions.

Yet however stimulating molecular biology has been to historians and philosophers, the most fascinating debate has been among scientists themselves. Has the influence of molecular biology been good on biology as a whole? You will find both positive and negative answers in the articles that follow. Has molecular biology really done nothing for medical science as Burnet and many others would claim? Has it so distorted the funding of medicine that competent medical science is at risk, and has it practically destroyed certain fields of research, such as human nutrition, to which it is very difficult to attract first-rate talent? Is the pursuit of a Nobel prize through molecular biology the ultimate aim of every budding biologist? So the muttering goes, both in public and in private. These are not questions for quick answers; they are ones, however, that those outside biology should be aware of, for they represent the very central issues of science and science policy and, who knows, may crop up anywhere else at very short notice. It only needs the right sort of paper in *Nature* to start it all off.

100 years ago



DR. LYON PLAYFAIR, C.B., has given notice that, on the House of Commons going into committee on the Education Estimates, he will call attention to the deficient ministerial responsibility under which the Votes for Education, Science, and Art are administered, and will move for a Select Committee to consider how such ministerial responsibility may be better secured. We believe that Dr. Lyon Playfair's views are strictly in accordance with those of the best scientific men of the country, namely, that the only satisfactory way of dealing with the subject will be by the appointment of a Minister for Education, Science, and Art.

From *Nature*, 9, 511, April 30, 1874.

Should scientists be seen and not heard?

In recent years there has been a marked change in the way the British government is advised about scientific affairs. John Gribbin discusses this change, and the position of science in industrialised society today, in the light of the recent appointment of Dr Robert Press to coordinate the government's scientific advice.

WITH the departure of Sir Alan Cottrell to become Master of Jesus College, Cambridge, the post of Chief Scientific Adviser to the government is not to be filled from the outside world of science. Sir Alan had held his post with a grade of second secretary in the Cabinet Office, and as such ranked above almost all the scientific advisers in the various departments and ministries (the exception being Sir Hermann Bondi at the Ministry of Defence). Responsibility for coordinating scientific advice within the Cabinet Office now rests with Dr Robert Press, who as Deputy Secretary (Science and Technology) holds the same rank as most of his colleagues who are the advisers to the individual departments.

Dr Press will no doubt carry out his new role as well as anyone but the change is bound to be seen by many as a decline in the status of the post; Dr Press has also commented that he will be continuing with his previous work—chiefly concerned with nuclear affairs—so it seems that the post is not regarded as a full time occupation. And the decline of the post of Chief Scientific Adviser to the government cannot even be said to be sudden. Sir Alan himself was in many ways in a less powerful position than his predecessor, Sir Solly (now Lord) Zuckerman, and was never, for example, the Chief of the Scientific Civil Service. The latest move could well be seen as a sign that the previous situation, under Zuckerman, has now been reversed.

This is a new situation which deserves particularly close attention since so few members of the government—or indeed of either House—have any training in scientific or engineering matters. In the past, it has certainly proved possible for an all-powerful Civil Service to advise and persuade governments without taking into account the best scientific considerations; this may not happen now, but it would be much less likely to happen if there was, once again, a man in the centre with the experience and personality to take a firm grip.

No doubt the argument will be put forward from some quarters that there is no suitable man to hold such a position, and that therefore the government has been forced into the present situation. That argument carries some weight; there are always people to be found with forceful personalities and a desire to tell the government what to do about science but alas that is not sufficient qualification. What is needed is someone from the scientific community who is both well informed and respected by that community, but who has the strength of character to deal firmly with the community when necessary.

Another argument which was put forward after the announcement of Dr Press's appointment was that it is in line with the Rothschild proposals for the organisation of

civil science in Britain. Now that there are Chief Scientists in all the essential departments, so the argument runs, there is no need for anything more than a coordinator in the Cabinet Office. That argument, however, seems to rest on a singular interpretation of the Rothschild report.

But outside the immediate issue, the question of the place of science in society is even more disturbing. At present there is an urgent and growing need for science to be used constructively by governments; there is a strong likelihood that the present situation will be no different in practice from the situation with Sir Alan Cottrell as Chief Scientific Adviser, but there is very little chance of changes being made in the required direction. In part, to be sure, the need is for a cleaning up of the mess made by the misuse of science in the past. That, however, makes the present need for correct use of science no less urgent. Yet there seems to be a great public apathy about science, at least in the industrialised nations of the world.

There is a need to provide scientific education in the broadest sense, to get the scientific message across to the general public and to the rank and file Members of Parliament, if not to the government itself. There is at present a bizarre situation in which there is concern at all those levels that the few millions of pounds worth of aid which Britain is providing for drought-stricken regions of Africa may be inadequate, and yet there is no move to provide a few tens of thousands of pounds for research into the causes of such droughts (see *Nature*, 248, 466; 1974). Seldom can there have been a more straightforward example of prevention (if possible at all) being better than cure; there must be many other cases where foresight and the correct application of current scientific knowledge would produce enormous benefits.

Part of the blame for the confusion and apathy in the public mind must be laid at the door of the Press. There were only a few brief paragraphs in the British national papers commenting on the new situation in the Cabinet Office, and at least one responsible newspaper carried an incorrect and misleading account which largely obscured the real significance of the situation. Members of both the Royal Society and the Institution of Mechanical Engineers have recently expressed concern about the failure of scientists to get their message across. If this concern is to be turned into something more constructive, those august bodies might well be advised to investigate ways of encouraging the spread of the scientific message through the journalistic medium.

It is, however, less easy to see an obvious immediate solution to the problem of providing the right kind of scientific advice to the government. If there are serious objections to placing too much scientific authority in the hands of one man, there is one obvious alternative which has been touted, and that is to expand the role and responsibilities of the Advisory Board for the Research Councils (ABRC). This has superficial appeal. Sir Alan was, of course, represented on the ABRC and Dr Press will be too. So the channel of communication is there and might be strengthened into something more without great difficulty.

But government by committee is always undesirable and advice by committee hardly less so. Since the role of Chief Scientific Adviser, which has now disappeared, was largely one of assessing priorities, it is difficult to see how the work could be delegated and shared out. Someone must see everything in order to assess the priorities, and in that case the committee is redundant. There seems no easy solution; but the present situation is far from satisfactory. At a time when the government is faced with questions of greater scientific complexity and urgency than ever before in peacetime it is disturbing to find the scientific community asking: Where is the voice of science and engineering in government?; it is perhaps even more disturbing to guess the likely public response to such a question: We don't know and we don't care.

international news

EACH year in the United States between 100 and 1,000 people undergo a highly controversial surgical operation which consists of the destruction of tiny portions of their brain. Popularly known as psychosurgery and designed to alter behaviour, the operation has become the centre of a bitter public debate over its legal and ethical implications, as a result of which the federal government is cautiously moving toward adopting a set of recommendations and regulations for controlling the technique.

The latest move is that staff members of the National Institute of Mental Health (NIMH), the agency which funds the bulk of government-supported psychiatric research, have sent a report to the Assistant Secretary for Health listing a set of recommendations for consideration as official government policy. As set out in a memorandum signed by the Director of the NIMH, Bertram S. Brown, and prepared with the help of a panel of distinguished outside experts, the suggested regulations would outlaw some of the more controversial applications of psychosurgery—its use on children, prisoners and incarcerated mental patients—but they stop well short of calling for a complete ban on the technique.

The controversy generated by psychosurgery has stemmed largely from charges that the technique is nothing more than a dressed-up version of lobotomy, the surgical operation popularised in the 1950s which has since been discredited, leaving thousands of people with impaired mental function. Supporters of the technique argue, however, that it is an acceptable form of therapy for severe behaviour disorders which have not responded to more conventional psychiatric treatment.

Unfortunately, the debate has not been helped by the fact that many of the psychosurgery operations carried out in the United States have been performed with hopelessly inadequate follow-up procedures, so that it is difficult to form any conclusions about the efficacy of the technique. As the NIMH memorandum puts it, "inadequacy of pre- and post-operative behavioral and psychological testing, lack of long term follow-up of patients, and general inadequacies of clinical and behavioral reporting characterise much of the published literature."

On the other hand, critics of psychosurgery have managed to cite examples in which psychosurgery operations are

Rules for psychosurgery

Colin Norman, Washington

Brown: signed memo



alleged to have resulted in severe damage to the intellect of patients and they have brought up passionate arguments to suggest that the operation has been used to alter the behaviour of emotionally disturbed children for the convenience of their parents.

One incontrovertible aspect of the operation is that its effects are irreversible because it involves the destruction of brain tissue which is not regenerated. In that case, why did the NIMH not recommend at least a moratorium on the technique until some of the more serious charges that have been levelled against the operation are cleared up?

The memorandum gives three reasons. First, such a recommendation "would constitute an unprecedented federal prescription of the parameters of permissible and impermissible surgery for the medical profession". (The NIMH staff did not feel themselves to be under such a constraint in recommending that the technique should not be used on children, prisoners and involuntarily detained mental patients, however.) Second, since there is no precise definition of psychosurgery, the memorandum suggests that a moratorium would be rendered ineffective because the operation could be performed under the guise of treatment for epilepsy and other neurological disorders. And third, the proposed regulations would at least amount to a partial moratorium on the most controversial forms of psychosurgery.

In short, the memorandum suggests regulations "with the intent of providing the maximum possible protection for potential psychosurgery candidates without unduly inhibiting practice for those cases which, judged by our present standards and knowledge, appear to require psychosurgery for relief of extreme mental illness or behaviour disorders". So the NIMH at least accepts some of the arguments for psychosurgery.

Perhaps the most important recommendation is that psychosurgery should be regarded as strictly experimental and not a form of therapy. Such a designation would slap a number of controls on use of the technique, such as the development of comprehensive research protocols to ensure that maximum scientific value is gained from each operation. It would also mean that psychosurgery should only be carried out in hospitals attached to universities and "every effort must be made to ensure that all reasonable alternative therapies . . . are attempted to an adequate extent before resorting to psychosurgery".

The NIMH is also recommending that a widespread effort be initiated by the federal government to obtain more precise information about the results of psychosurgery operations performed in the past, the idea being that such information can later be used as a basis for more permanent guidelines governing the future use of the technique.

The memorandum is now being reviewed by Charles C. Edwards, Assistant Secretary for Health, but whatever finally becomes of it, the federal government can only directly control those psychosurgery operations performed with government money. The control of such operations carried out in hospitals and universities independently of federal funds lies outside the direct jurisdiction of the Department of Health, Education and Welfare.

But at least the widespread public controversy which has been generated in the past couple of years, chiefly through a court case which halted the use of psychosurgery on an involuntarily detained mental patient last year and a bruising public inquiry conducted by Senator Edward M. Kennedy's health subcommittee, should make it more difficult for the operation to be carried out with inadequate controls.

Pure science and the energy problem

Miranda Robertson, Chicago

MUCH the largest contribution of the Argonne National Laboratory to the problem of developing a new energy technology is its experimental liquid-metal fast-breeder reactor, and nuclear energy will continue to account for nearly half the total research expenditure of the laboratory. But recent shifts in the emphasis of federal funding are reflected in the expansion of research on a wide range of alternative and accessory approaches to the problem. At the same time, the laboratory (which is controlled by the United States Atomic Energy Commission) is beginning to feel the effects of a general movement to involve basic scientists in applied research, as interpreted by its new director, Dr Robert G. Sachs, now at the end of his first year at Argonne. A vigorous proponent of the view that fundamental scientists represent a national resource to be supported for reasons of enlightened self-interest, he believes that the time has now come to draw on that resource for the answers to a national problem. He also believes that a degree of mission-orientation need detract from nobody's fundamental research pursuits. The effects of both federal and local policy are nowhere better illustrated than in the birth of a new research project on solar energy in which Argonne personnel are collaborating with Dr Roland Winston from the Enrico Fermi Institute at the University of Chicago, where Dr Sachs holds a chair.

The outcome of this joint enterprise could be the development of a much more efficient solar energy collector than could be envisaged with known techniques; but it began as pure research on high energy physics. The problem to which the Winston advice was originally addressed was that of collecting Cerenkov radiation for the detection of rare electrons resulting from the β decay of a λ particle. The essential feature of the device was that it should not miss any radiation from the reaction.

Eventually, by departing from the principles of imaging optics, which limit the possible degree of concentration of the intensity of solar radiation to a factor of three, he arrived at an ideal light collector in the form of a cylindrical paraboloid with a theoretical maximum degree of concentration of ten times. The device represents a new principle as far as modern physics is concerned, although Professor Ricardo Levi Setti, a colleague of Winston's in the physics department, has discovered identical

shapes in drawings of Descartes and in the individual eye-cups of the horse-shoe crab. What Levi Setti also recognised, however, was that as well as having an interesting past the device has an important potential future as a concentrator of solar energy.

The problem of concentrating sunlight is only one of the obstacles to the utilisation of solar power to generate electricity, but it is one of the principal stumbling blocks in using it to heat and cool individual homes. The exploitation of solar energy for this purpose is already feasible and as a result of vigorous advocacy in the past year there has been an increase in the allocation of research funds as well as the institution of tax incentives for the conversion of buildings from conventional heating and cooling systems. But although solar heating is comfortably within the capacity of existing types of collector, cooling, which is much less efficient and in some areas in the summer stretches electricity generation to the point of overload, is more marginal. The increased concentration factor of the Winston light collector may overcome the difficulty. Its other

important advantage is that it can be designed so as to obviate the need for tracking the movement of the Sun across the sky, thus greatly reducing the complexity and the energy cost of the system. Winston's first model for a roof unit consists of a series of collectors shaped as paraboloid troughs which can be aligned with the Sun's trajectory so as to collect light for an average of eight hours a day.

The promise of the device has been endorsed by the allocation, in mid-fiscal year, of some \$200,000 from the United States National Science Foundation and the Atomic Energy Commission for feasibility studies. Winston and his collaborators at the Argonne are now working on such questions as how much light is actually collected by an ideal light collector made from nonideal materials and functioning under nonideal conditions. One of the problems being tackled is the development of what amounts to a new meteorological dictionary: for example, 'overcast' might mean something very different in terms of collectable solar radiation than it means in the week-end weather report.

Solar energy in suspense *Colin Norman, Washington*

Although almost everybody agrees that solar energy is a splendid idea whose time has come, a bill designed to hasten commercial application of solar heating and cooling technology has fallen foul of a jurisdictional squabble in the United States Senate and its passage has been delayed for several months while no less than five different committees have staked their claims on the legislation.

Passed by the House of Representatives in mid-February, the bill is designed to provide federal support for the development and testing of solar heating and cooling devices in private homes, office buildings, factories, schools and various other buildings. The idea is that the National Aeronautics and Space Administration (NASA) would be given some money to let contracts with industry for development and mass production of solar heating and cooling units, while the Department of Housing and Urban Development (HUD) would be responsible for carrying out prototype demonstrations to see how well the units perform in everyday use.

After being approved by the House—by a margin of 248 votes to two—the bill was referred to the Senate Committee on Aeronautical and Space Sciences which made a few amendments and passed it on March 13. In the meantime, how-

ever, four other senate committees—Commerce, Labour, Interior and Banking, and Housing and Urban Affairs—all argued that the bill should come under their purview, so it was then referred to each of them separately.

The irony is that once the various committees have finished guarding their legislative jurisdictions, the bill which finally emerges will probably be similar to the version which was passed by the House, except for one important detail. The House bill would have provided \$50 million for the programme over the next five years, but the Senate Space Committee has suggested that NASA and HUD should each get \$5 million next year to carry out their parts of the enterprise and funding for future years should be left open for now.

As for the administration's attitude to the bill, Dr H. Guyford Stever, Director of the National Science Foundation and Science Adviser to the White House, has argued that although the demonstration scheme is a good idea, it is a little premature. The Administration has already proposed that \$50 million should be spent next year on solar energy research, and Stever reckons that it would be wise to wait until some of this effort bears fruit before rushing into a federally sponsored application programme.

The Strangelove scenario

Colin Norman, Washington

BY THE year 2000 there could be enough plutonium and enriched uranium flowing through the nuclear power industry in the United States to manufacture about 250,000 crude atomic bombs. The sheer magnitude alone is sufficient to conjure up Dr Strangelove scenarios of nuclear theft. But is it really feasible for a few terrorists to make off with several kilograms of the stuff, fashion it into a weapon and hold a city to ransom—or even to create a nuclear holocaust in the Middle East?

According to a book-length report prepared for the Ford Foundation's Energy Policy Project, the short answer is yes, unless the Atomic Energy Commission (AEC) improves its control over weapons-grade nuclear material and unless officials in the energy industry, many of whom are "only vaguely aware of the problem", are made to take effective (and costly) steps to safeguard the potential nuclear weapons that they handle every day. (*Nuclear theft: Risks and Safeguards*. Mason Willrich and Theodore B. Taylor. Ballinger Publishing Company, Cambridge, Massachusetts, 1974).

The study's conclusions are likely to provide some powerful ammunition to nuclear critics in the United States who have recently seized on the issue of weapons' proliferation as yet another club with which to beat the AEC and the nuclear industry, particularly with respect to plans to build a string of plutonium-producing fast-breeder reactors around the country. But they are unlikely to shake the Administration's unswerving commitment to nuclear power, and spokesmen for the AEC have already rebutted the report's chief conclusion by arguing that existing safeguards are sufficiently stringent to ensure adequate protection of public safety and national security.

Be that as it may, the study provides strong evidence to suggest that a crude atomic bomb could be manufactured in a clandestine laboratory by a few people with a relatively modest amount of nuclear know-how, about 10 kg of plutonium oxide, a substantial amount of chemical high explosive and "materials and equipment that could be purchased at a hardware store and from commercial suppliers of scientific equipment for student laboratories". A bomb made of highly enriched uranium (containing more than 90% uranium-234) or uranium-233 would be assembled a little differently from a plutonium bomb, but it could also be made relatively easily with a supply of

15 kg or more of fissionable material.

Although that may sound like fantasy to some weapons designers, the authors of the study point out that few experts have asked themselves the question: "What is the easiest way I can think of to make a fission bomb, given enough fission-explosive material to assemble more than one normal density critical mass?" And they cite an article in the *Encyclopedia Americana*, written by Dr John S. Foster, former Director of Defense Research and Engineering and an expert on nuclear weapons' technology, who states that "the only difficult part of making a fission bomb of some sort is the preparation of a supply of fissionable material of adequate purity: the design of the bomb itself is relatively easy".

Moreover, the two authors of the report have some impressive credentials in the nuclear weapons field to back up their analyses. One of them, Mason Willrich, now Professor of Law at the University of Virginia, was General Counsel of the United States Arms Control and Disarmament Agency, and the other, Theodore B. Taylor, worked on nuclear weapons design at the Los Alamos Scientific Laboratory and was Deputy Director of the Pentagon's Defense Atomic Support Agency. Taylor emerged a few years ago as the chief critic of the AEC's nuclear safeguards and has become a rather prominent thorn in the agency's flesh.

It is not difficult to predict the consequences if a crude nuclear weapon does end up in the hands of a terrorist organisation. The authors of the study point out, for example, that even a "small" nuclear device exploded underneath a large city skyscraper might kill as many as 50,000 people: a comparable explosion in the centre of a football stadium could lethally irradiate about 100,000. As for the injection of nuclear weapons into the Middle East, the political fallout would be devastating.

So, assuming the validity of the theory that a crude atomic weapon could be manufactured relatively easily, the crucial consideration is how easily can fissionable material be stolen from the nuclear power industry? Willrich and Taylor argue that although there is little risk at present that a terrorist could make off with a few critical masses of explosive material, the burgeoning growth of nuclear energy and the changing characteristics of power plants will greatly increase the chance of illicit diversion of the stuff. They suggest that unless the AEC adopts an improved safeguards system now, it may soon be too late.

The most vulnerable part of the fuel cycle to terrorist activities is the transportation of weapons-grade materials

between facilities, according to the study, and the greatest chances for diversion will come when large quantities of plutonium (in particular) are moved around the country. At present, the movement of plutonium is not substantial, but in the next few years it is likely to grow significantly.

Nuclear energy is now produced almost entirely by light water reactors which are fuelled with uranium, only slightly enriched with the uranium-235 isotope. The fuel itself is thus of no use as weapons material, but the reactors do produce plutonium, which is separated out from the spent fuel rods and stored. According to Willrich and Taylor, this plutonium is now adequately protected against theft, but in the late 1970s it will be recycled for use as fuel in light water reactors, which means that it will be transported from the storage facilities to the power plants. The inherent risk of theft will then be "considerably greater".

In the 1980s and 1990s, when the liquid-metal fast-breeder reactor (LMFBR) is likely to be introduced commercially, the availability of weapons-grade nuclear material will again increase dramatically. The LMFBR produces large quantities of plutonium, which will be extracted from the spent fuel rods and recycled for use as reactor fuel. Since the AEC has forecast that by the year 2000 breeder reactors could be generating up to 750,000 MW of electricity, Willrich and Taylor calculate that if all goes according to plan, about 3,000 truckloads of plutonium oxide would be shipped to LMFBR and light water reactor fuel fabrication plants every year.

And these figures are given added significance if the possibility that a small amount, insufficient to make a bomb but enough to make a radiological weapon, is taken into account. Since plutonium is highly toxic, and easily dispersed in the form of tiny airborne particles, it would be possible to pose a severe risk to a large population with only about 100 grams.

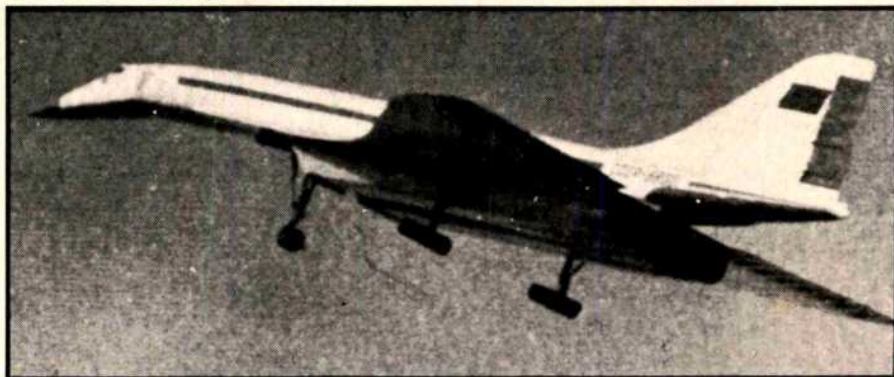
Given the possibility of such dire consequences resulting from the theft of comparatively tiny amounts of fissionable material, is the AEC making sure that the material is properly protected? Willrich and Taylor think not. For one thing, they point out that there are no specific requirements for the physical protection of less than 2 kilograms of plutonium, although that would be more than enough for a radiological weapon. And for another, they suggest that regulations governing communication with vehicles transporting fissionable material are not particularly effective.

But a safeguard system can be developed to keep the risk of theft to "very low levels". First, the study recom-

mends that the AEC should design a system of safeguards for each nuclear fuel cycle, based on the best technology available, and the agency should also consider the possibility of establishing a special federal security service whose sole job would be to protect nuclear material at fixed sites and during transit. Willrich and Taylor also suggested that careful consideration should be given to locating fuel reprocessing and fabrication plants side by side, to cut down the transportation of nuclear fuels between facilities. Finally, they suggest that the United States government should initiate discussions with other countries which have substantial nuclear power programmes in an effort

to develop a common policy towards international safeguards against nuclear theft anywhere in the world. The AEC's reaction is that its safeguards are more effective than Willrich and Taylor suggest.

But the credibility of the agency's safeguards received a severe blow last year when the General Accounting Office (GAO), a watchdog agency of the United States Congress, published the results of an inspection of three plants which handle strategically significant quantities of fissionable material. The GAO found evidence of laxity such as gaping holes in perimeter fences, inadequate burglar alarms and vulnerable storage facilities.



The TU-144: traumatic crash

Russians in the air *from our Soviet Correspondent*

AVIATION has always been considered a 'prestige' achievement in the Soviet Union, from the early 1920s when the propagandists of atheism solemnly argued that "There is no God for our brave airmen have failed to find him," through the spectacular air-lift, in March-April 1934, which evacuated 104 icewrecked survivors (including the inevitable newborn infant) from the polar research ship 'Semen Chelyuskin', through the 1945 anniversary celebrations of the Academy of Sciences when Joukowski received almost as much acclaim as Stalin up to the present day and the maiden flight of the TU-154 jet airliner from Moscow to Helsinki on April 9. The highly publicised crash of the TU-144 at last year's Paris air show, therefore, must have been a traumatic experience, not only for the Soviet aviation industry but for all concerned in the mass media.

According to the tenets of Socialist Realism, the press and other media, including the fine arts, should strive to present, not the transient defects of the world as it is but a sort of Platonic ideal of what it one day will be. The aftermath of the TU-144 disaster has, therefore, resulted in an even greater than usual press coverage of the positive aspects of aviation.

Nevertheless, since the Paris disaster there seems to have been a change in Soviet aviation policy, as reflected in

the press coverage. Whereas in February 1973, an *Izvestiya* article could state that the decision of PanAm and TWA not to take up options on Concorde was dictated not by commercial but by political motivation (since the United States "have nothing to compete with Concorde or the TU-144"), now the stress is all on co-operation with the United States. In January, a five-year cooperation agreement was signed between Lockheed and the USSR State Committee for Science and Technology, providing for the joint development of civil aircraft construction, navigation systems and aviation electronics. The inauguration of the direct Moscow-Washington air route was hailed by *Pravda* as "a major contribution to the further development in the extension of mutually advantageous economic cooperation of two great powers". For the moment, the negative aspects of competition seem to be minimised.

And yet since Concorde (in conjunction with the TU-144) has always been presented in the Soviet press as Europe's answer to the American aviation "threat", it is perhaps not without significance that, according to the Novosti agency, a factory at Kramatorsk (Ukrainian SSR) has begun work on a 60,000-tonne hydraulic press for the manufacture of large scale components of Concorde supersonic airliners.

OECD probing in Australia

Peter Pockley, Sydney

A TEAM of three examiners from the Organisation for Economic Co-operation and Development (OECD) is now in Australia for the first independent examination of the nation's organisation, funding and policies for science and technology. The team is led by Dr Alexander King, the OECD's Director-General for Scientific Affairs, who is an old hand at OECD science policy surveys; this will be his last one before retirement. Other members of the panel are Dr Freidrich Schneider of West Germany and Dr J. Wautrequin of Belgium.

Australia joined two dozen other nations as a member of the OECD just over two years ago and the current survey of Australia is the nation's first major examination under the organisation's critical microscope. The results should be interesting, not only for scientists but in a wider sphere as a measure of the nation's maturity in taking authoritative, international criticism on the chin and of the government's capacity for instigating sensible, perhaps radical, reforms which have not originated from internal, political pressures.

That such reforms may be recommended seems likely from the widely circulated comments of the OECD panel before its arrival in Australia at the end of March. Its comments were written in response to the background document prepared for the OECD by the Department of Science; they spoke of a good deal of thought being needed about weaknesses in the system, lack of balance and the need to develop in new directions. The OECD also noted the heavy concentration on government-run or government-sponsored science.

In a closely packed series of meetings, the team is now finding out for itself just how decisions of priority are made (or more to the point, how they are not made) in Australian science. Given the level of expenditure on research and development in Australia (now running at about \$A450 million a year), it may seem surprising that no similar survey has previously been conducted within Australia. Even now, there is no academic unit or independent institute in Australia which is professionally interested in the study of science policy on anything more than a part-time basis. The School of Sociology at the University of New South Wales is the nearest approximation to such a unit; senior staff are making a habit of spending sabbatical years in Britain at the Science Policy Research Unit at the University of Sussex.

Another continuing deficiency which the OECD team has already picked up, is the small amount of information in general circulation about the achievements and quality of scientific research *per se*. The background document presented by the government to the OECD confirmed this by dealing exclusively with the organisation of science in the country, almost as if the products of the research effort were hardly worth mentioning. The OECD examiners are hardly likely to be diverted from their stated objective of assessing the value of work done, as measured through accepted international criteria of scientific interest and merit. Their report should therefore prove doubly informative to Australians in positions of power who are ignorant of the very real achievements of science, notably outside the fairly well publicised government sector, such as the Commonwealth Scientific and Industrial Research Organisation (CSIRO).

In the week before the OECD team arrived in Australia, the Minister for Science, Mr Bill Morrison tabled a Green Paper in the Parliament entitled *Towards an Australian Science Council*. Presented as a discussion paper rather than a blueprint, this document spells out the government's long-drawn-out uncertainty about the terms of reference and functions of the council, which they first proposed in their Labour Party Platform before the elections of December 1972.

In his speech about the green paper, Mr Morrison opened with a statement about "public disenchantment (which) has risen with the part played by science and technology in developments of dubious value to mankind". While this point can obviously not be ignored, the prominence given to it by the minister does cast doubts on his determination to bat vigorously in Cabinet on behalf of an expanding budget for science. All present indications are that funds for science will remain largely stagnant in relation to many other rapidly expanding areas of government spending in the country.

In this respect it is possible that the OECD examination is being quietly considered as providing justifications for different ways of slicing up the same financial cake. But, the OECD has made many acute observations in other countries, and it is equally possible that it will shake the assumptions of the Australian scientific bureaucracy and its political masters to the core.

Unfortunately, though, the effect of the report may be dulled within Australia by the present plan to present and discuss the report at the OECD headquarters in Paris, rather than in Australia.

European Earth scientists disunite

Peter J. Smith

ONE of the benefits often claimed for the recent revolution in the geological sciences is that it has finally succeeded in uniting solid-earth scientists of all disciplines. The new global tectonics, the argument goes, has provided a conceptual framework which enables almost every student of the Earth to find a place in a common enterprise. In the purely academic sense there may be some truth in it; but if recent events in Europe are anything to go by, interaction between geologists and geophysicists remains firmly bound between the covers of the learned journals.

The possibility of a serious breakdown in communication between the two groups first became apparent late last year when the General Secretary of the European Geophysical Society (EGS) discovered more or less accidentally that the Geological Society of London and the University of Reading were jointly planning a "meeting of European geological societies". For some time the precise purpose of the meeting remained unclear to the geophysicists, although it was widely believed that in addition to scientific sessions there would be an evaluation of the need and/or desire for the formation of a Geological Society of Europe (GSE). (One of the meeting's chief organisers said that no such thing was intended, although when the EGS General Secretary later relayed this information in good faith to a scientific conference in Peru it was flatly contradicted by another of the meeting's organisers).

Now that it has been formally announced that the meeting will take place at the University of Reading in September 1975, it is possible to see that its official object is "to provide an opportunity for members of European earth science societies to discuss (1) common scientific, economic and organisational problems, and (2) ways of promoting closer cooperation in future". The vagueness of this statement and the continued inability of the EGS General Secretary to get any more specific information from the sponsors of the Reading meeting have apparently upset many members of the EGS Council who feel that, whatever is going on, some prior consultation would have been both desirable and tactful. They are also concerned about the scientific theme of the proposed meeting, which is "Europe from crust to core". There being no such thing as the geology of the core, it is evident that the Reading meeting must incorporate some element of geophysics—

a subject which is already catered for on a Europe-wide basis by the EGS itself.

In spite of previous contradictory statements it seems likely that the possibility of a GSE will be discussed at Reading in some form. What particularly puzzles the EGS Council, therefore, is that the sponsors of the meeting apparently see no merit in drawing on the experience of the founders of the EGS and the council itself. After all, for several years prior to the formation of the EGS the problems involved in the cooperation of European earth scientists were discussed in great detail.

Given that European cooperation is desirable, the basic question as the geophysicists see it is whether there should be a single European Earth Science Society or whether geologists, geophysicists, geochemists and other such groups should go their own separate ways. Both arguments and individuals within the EGS seem to be about equally divided on this point. It is widely agreed that of overriding importance is the desirability of greater communication between earth scientists of all disciplines, a need increasingly dictated in any case by the way the subject itself is developing. On the other hand, if that implies the need for a single organisation, there are likely to be severe practical problems.

For one thing, geologists greatly outnumber the members of the smaller disciplines, and so there is a real danger that in an all-embracing body the latter group would become submerged—a thought which was very much in the minds of the founders of the EGS when the question of scope first arose. Second, there is the problem of logistics. More than 600 scientists attended the first EGS meeting held last year in Zurich; several thousands could be expected at a meeting of a combined European Earth Science Society. Quite apart from the fact that many people have expressed abhorrence at the mere idea of such a large gathering and that others have doubted the possibility of true communication in such conditions, suitable venues for very large meetings in Europe are surprisingly scarce. Moreover, one of the prime aims of the EGS in particular is to attract relatively impoverished students and research workers at the beginning of their careers; and this cannot be managed without cheap accommodation for conference participants. Finally, there is the point that there are still many geologists and geophysicists working in fields in which, rightly or wrongly, there is still little recognised need for cooperation with other branches of the Earth sciences.

The EGS believes that if a single European organisation with a single

annual meeting is to be ruled out, the best solution would involve individual bodies holding separate meetings at the same location but in successive weeks. In this way scientists could attend one, or the other, or both, of the meetings as they wish without doubling travel costs. The fact remains, however, that no satisfactory solution will emerge at all and chaos is likely to result unless geologists and geophysicists talk to each other. The EGS Council has now written to the organisers of the Reading meeting expressing concern at the meeting's scientific theme and formally requesting consultation on matters of mutual interest.

New union in Canada

from a Correspondent

ON February 22, 1974, the first meeting of a newly formed Canadian Geophysical Union (CGU) was held in Ottawa; it was chaired by the union's first president, Professor J. Tuzo Wilson of the University of Toronto. Canadian geophysics has been well served in the past by the National Research Council of Canada (NRCC) through its system of associate committees and sub-committees. Established in 1945 (also under the chairmanship of Professor Wilson) and active ever since, the Associate Committee on Geodesy and Geophysics has been the main co-ordinating body, the clearing house, for scientific and technical information, and the benevolent patron of most geophysical research in Canada. The main activities of the Associate Committee were semiannual meetings at which the progress of various research projects were discussed and early scientific results announced, and the publication of an annual *Geophysical Bulletin* containing the reports of activities of all important centres of geophysical research in Canada. Twenty-five published volumes of this bulletin provide an opportunity for all geophysicists to find out about programmes of research outside their own field. But the NRCC itself is being reorganised and as part of this process geophysicists have been nudged out of their warm and cosy nest.

The programme of the inaugural meeting consisted of two parts: a presentation of "Frontiers of Science" lectures and a panel discussion on the future of geoscience in Canada. The first part suggested that the progress of geophysical research is healthy; the second part suggested that the assembled membership did not anticipate any serious problems ahead. Surprisingly, there was no mention of shortages of research funds although there were polite suggestions that more effort

should be devoted to this or that project (theoretical geophysics, geodynamics of the Earth's interior, exploration geophysics research and so on). The question of whether the participation of Canadian geophysicists in international science is at an adequate level was discussed but no conclusions were reached. Also unanswered was the question: "What are the government organisations responsible for regional geophysical surveys going to do when these regional surveys have been completed?"

The CGU will be the national body adhering to the International Union of Geodesy and Geophysics (IUGG). Its stature at the international level will be assured by the support it has from prominent Canadian geophysicists. The strength of its voice in national affairs will depend on the membership it can attract and there will be competition with a number of specialised societies loosely federated in the Canadian Geoscience Council. Because of the size and the geographical situation of the Canadian land mass and the adjacent offshore areas, geophysicists working there have always taken their responsibilities to international science seriously. On the continental scale these responsibilities have included standardised seismological networks, regional gravity maps, surveys of the magnetic pole and research on Aurora. On the scale of global geophysics the new union has an important role to play and the international scientific community will watch its growth with interest.

Medical research in the dock

A Boston grand jury has set the stage for two separate court battles, one of which holds important implications for medical research, and the other involves a legal challenge to a key aspect of the historic decision on abortion which was handed down last year by the US Supreme Court.

In the first case, four medical researchers have been accused by the Grand Jury of violating an obscure Massachusetts law in connection with a research project they carried out at Boston City Hospital in 1971 and 1972. If the accusation stands up in court, it could put an end to all research on foetal tissue in Massachusetts.

The research involved giving antibiotics to 33 women scheduled to undergo therapeutic abortions, in an attempt to determine which of the drugs was more effective in crossing the placenta. The objective was to find an antibiotic to use instead of penicillin for curing foetal infections. After the abortions were performed, the dead foetuses were

analysed for signs of drug residues.

The doctors have been charged with illegal dissection of non-living tissue under a 19th century Massachusetts law designed to prevent graverobbing. Medical researchers in the Boston area have been quick to point out that research on dead foetal tissue has played an important part in the development of vaccines and that foetal tissue is often vital for all sorts of medical investigations. In fact, two Harvard scientists, Dr Thomas Weller and Dr John Enders, won the Nobel Prize in 1954 for growing polio virus in cells cultured from foetal tissue.

If the prosecution is successful, the ban on such research would extend only to Massachusetts, but since there is a huge medical research complex in the Boston area, its effect would be very keenly felt. In the meantime, the Massachusetts State Legislature is considering a bill which would impose a flat ban on research involving aborted foetuses, so even if the ancient graverobbing law proves to be ineffective, Boston scientists are still in danger of being saddled with legal prohibitions on their work.

The second case involves an indictment against Dr Keith Edelin, Chief Resident in Obstetrics and Gynaecology at Boston City Hospital, who has been accused by the same Grand Jury of manslaughter in connection with a legal therapeutic abortion which he performed last October. He has been charged with killing a foetus, reported to be between 16 and 24 weeks old, which the prosecutor's office maintains was a viable human being.

When the case is eventually brought to court, the central issue will revolve around the responsibility of a doctor to do all in his power to keep an aborted foetus alive. This is an issue which was left rather murky by the Supreme Court, which argued that the state has a right to protect the life of an unborn child only when it is 'potentially able to live outside the mother's womb, albeit with artificial aid'. This usually occurs, the court said, at 'about seven months'.

The Supreme Court decision overturned a Massachusetts anti-abortion law, and Edelin's indictment has been viewed by some observers as an attempt to challenge the court's decision and to define more rigidly the concept of viability. In Catholic Boston, there is a very potent anti-abortion lobby.

Edelin's colleagues at Boston City Hospital have published a statement suggesting, in fact, that he may be a scapegoat for the anti-abortion forces, and they have predicted that he will be cleared when the facts are known. In any case, the court case is going to be closely watched throughout the country.

news and views

Water, myth and magnetism

IN these days of the Geller effect, the perhaps magnificently lost causes of quarks and gravity waves and the great international *Bockschiessen* over polywater, it would be a pity to lose sight of the fact that, at any one time, there are probably a handful of minor absurdities lurking on the fringes of orthodox physics which, although lacking some of the sensational character of the examples mentioned, seem nonetheless worth bringing into the open.

One such case recently drew me into previously unsuspected backwaters of the physicochemical literature to a number of articles which, though preposterous in many respects, seemed to leave enough important questions unanswered to be worth describing. It concerns the alleged possibility of producing mysterious changes in the physical properties of water by the application of relatively weak magnetic fields—permanent changes or transient ones depending which of several authors one is inclined to believe.

The subject of the magnetism of water surfaced briefly in the mainstream literature about two years ago with a perfectly unsensational paper by Quickenden, Betts, Cole and Noble of the University of Queensland (*J. phys. Chem.*, **75**, 2830; 1971) who carried out a meticulous null experiment showing, to nobody's great surprise, that no detectable changes in *pH* can be found in magnetically treated water, none being in any case remotely conceivable in terms of any electronic model for the water molecule. It is in the more obscure papers which inspired Quickenden *et al.* to undertake such a seemingly futile measurement that the case becomes curiously and curiously.

Their immediate stimulus was a paper by Joshi and Kamat (*J. Indian chem. Soc.*, **43**, 620; 1966) claiming to have obtained not only changes in *pH* of about half a unit in CO_2 -equilibrated water but—for good measure—distinct, irreversible changes in surface tension and dielectric constant as well, all with fields little stronger than that of a permanent bar magnet. At this point the armchair theorist (or rather one perched high on a Patent Office stool) finds that the subject extends back from familiar territory into the somewhat alien world of hydrology, boiler-feed management and the suppression of incrustations in the conduits of spas and watering places. It seems that, for a considerable time, boiler engineers in many parts of the world have been passing their feed water through quite mild magnetic fields and obtaining a sensational reduction in the deposition or, more correctly, the adhesion of scale as a result. A wave of testimony to this passed through the relevant literature about ten years ago, with rapturous accounts originating as far apart as the Works for Heavy Machinery Construction in Alma Ata (Bitny *et al.*, *Bezop. Truda v Prom.*, **3**, 22; 1959) and the Belgian Institute for Corrosion Research CEBELCOR in Brussels. Some accounts claim that passage of feed water through the field of a small bar magnet can actually lead to removal of scale already deposited, patents have been taken out and there is mention of a figure of 50,000 successful installations of the "CEPI" device, embodying a small permanent magnet in the inlet.

When I took this up with one particularly wise and ex-

perienced physical chemist, his reaction was that this was nothing compared with some of the almost magical practices resorted to by boiler engineers. For instance, had I heard of the use of glass globes containing helium and a little mercury tethered in the water supply and said to be almost as effective as the magnetic system? I had not but some further research turned this up too, in an article by Friedel (*Chem. Ztg.*, **84**, 539; 1960) who attempts to explain the electrochemistry of both this, the "Tonisator" as it is called, and the CEPI method. Friedel's explanations, invoking Geissler discharges and a permanent magnetically induced strain in the bond angles of the H_2O^+ ion, are far fetched by any account, though this might be held to be of little concern to the improbable 50,000 maintenance men the world over, released by the CEPI device from the unpleasant task of periodic chipping out with a descaling hammer.

But, if one must single out the most remarkable paper of all on water magnetism, it must surely be that of Bruns, Klassen and Kon'shina of the A. A. Skochinskii Mining Institute in Moscow (*Colloid J. USSR*, **28**, 129; 1966), likewise backed up by another whole sublitterature from Russian specialist sources. Bruns and colleagues go further than the other work cited in two respects. They claim that the optical absorption of water can be altered up to some 30% by quite moderate magnetic fields, the treated water regaining its normal state with a time constant of some hours, and further that the effect is periodic in the applied magnetic field strength. (Here, however, it must be solemnly recorded that some of the 'periodic' data presented is drawn with two maxima and minima spaced among only five data points.) The explanation proposed is that there is a 'resonance' interaction with the vibratory motion of groups of associated water molecules under the influence of the field . . .

After all this it seems almost hubristic to suggest that, if magnetic fields can really interfere with the *pH* or any other electrochemical property of water significantly, it might be a very painful and hazardous business putting one's finger between the poles of a strong electromagnet. (True that blood is effectively buffered, but why should the buffer *pKs* be any more immune to magnetic interactions?) Of course biological effects of magnetic fields are known but they are weak and elusive and, on the whole, poorly documented. Mice show no particular ill effects from a few hours at 150,000 oersted and men have been exposed to 20,000 oersted for a quarter of an hour or so. Meanwhile, at the invertebrate and cellular level, there is much dispute as to whether fields can cause abnormalities in *Drosophila*, sea urchin eggs, photobacteria and ascites tumour cells though certain creatures such as the mud snail *Nassarius obsoletus* seem candidates for strange susceptibilities. (For details of these experiments and much else see *Biological Effects of Magnetic Fields* (edit. by Barnothy, M. F.), Plenum, New York, 1964). Of the various marginal effects, magneto-tropism in plants seems the best established. From these matters it is but a short step to the very strange phenomena of dowsers and bird navigation and their attendant literature, as rich in speculation as short on physical theory. Hall effects and electron interactions in proteins, perhaps, but only by wildest stretch of the imagination (and the wild stretching of imagination is common enough in such fields) attribution to physical changes in water.

So what in the end of the contented boilerman in Alma Ata and the guidance system of the mud snail? Can these

conceivably have enough in common to warrant a new, close look at the magnetophysics of aqueous systems—or should physical chemistry, with uneasy glances over the shoulder at the polywater nonsense, let this particular sleeping dog lie? For it could well be that all the various effects have but one essential in common, their ability to elicit man's tendency, active since ancient times, to relate the mystery of the magnetic force and its field to almost every other enigma that troubles him. A tendency to which a remarkable variety of scientists seem in no way immune.

From our Molecular Physics Correspondent

Continuity of Precambrian life

ALTHOUGH Precambrian microfossils have been known for many years, little attention was given to this area of investigation until after the descriptions of the microorganisms from the 1.9×10^9 year old Gunflint Chert^{1,2}, part of the Lake Superior banded iron formations from Canada. These publications appeared in 1965 and initiated the examination of many ancient rocks by scientists of various disciplines in an endeavour to throw light on the origin and evolution of living systems. This great burst of interest coincided with the scientific activities associated with the preparation for the American manned space flights and lunar landings which renewed interest in the possibilities of extraterrestrial life, its origin and possible contamination of the Earth by returning astronauts.

The problem was tackled on a broad front. The chemists investigated both the soluble and insoluble fractions of the organic matter in Earth rocks, meteorites and, later, lunar rocks. The palaeontologists examined the rocks for evidence of morphologically preserved microfossils, and found these in Earth rocks. There have never been any indications in lunar rocks of structures possibly interpretable as biogenic in origin and the bizarre, chemically organic, six-sided box-like structures from the carbonaceous chondrites have been interpreted as condensation products of abiogenically produced organic compounds³. The small hollow, spherical organic bodies also recovered from carbonaceous chondrites have been similarly interpreted⁴, and it is significant that the surface texture of the meteorite spheroids is markedly different from the surface texture of spheroids from the 3.355×10^9 yr old Onverwacht Group rocks of South Africa^{4,5}.

A survey of the earlier work can be found in Schopf⁶. Three time intervals have received considerable attention: $3.4-3.0 \times 10^9$ yr; around 2.0×10^9 yr; and from 1.3×10^9 yr to the base of the Cambrian.

In the first of these time intervals occurs the oldest, well dated, easily accessible sedimentary succession, the Onverwacht Group of the Swaziland System of South Africa. The 3.750×10^9 yr old Amitsook and Issua rocks of Greenland are less accessible, and in the case of the Amitsook, highly metamorphosed. It would be interesting to see the results of the $\delta^{13}\text{C}$ analysis on the graphite from these rocks, although the high grade of metamorphism would make interpretation of the results very difficult.

Even in the Onverwacht Group, the rocks are part of a green stone belt, they have been folded and have suffered some degree of metamorphism⁵. Nonetheless, small (up to 20μ) spheres and filaments have been found in moderate abundance in the cherts, and their biogenicity has in general been supported by the evidence of the $\delta^{13}\text{C}$ ratios of the kerogen⁷. The kerogen itself has a chemical structure similar to that of kerogens produced by degradation of known biogenic compounds⁸. Controversy centres on the older part of the Onverwacht, the Theespruit Formation. The $\delta^{13}\text{C}$ ratios of its organic matter are anomalously low

and this may imply the presence of possibly pre-biogenic or at least pre-photosynthetic vital activity⁸. This difficulty cannot be resolved however, until the effects of metamorphism by heating and by pressure on $\delta^{13}\text{C}$ ratios are better understood.

Evidence for early photosynthesis is adduced from the discovery of stromatolites in the Rhodesian 3.1×10^9 yr old Bulawayan System⁹, that is roughly the same age as the Fig Tree Group which directly overlies the Onverwacht Group of South Africa. Microfossils in the Fig Tree Group were first discovered and named by Barghoorn and Schopf¹⁰. Although some doubt has been cast on the validity of the rod-like 'bacterial' forms discovered only by electron microscopy⁶, the spheres discovered in optical thin section are regarded as reliably biogenic⁷ and further investigation of these rocks is necessary.

Until very recently, a huge gap in knowledge existed between the Fig Tree spheroids and the banded iron formation microorganisms—a time gap of nearly 1,000 Myr—but two groups have recently described^{11,12} the discovery of extremely well preserved blue green algae in dolomitic limestones of the 2.3×10^9 yr old stromatolitic Transvaal System. These algae have a remarkably modern aspect and have been closely compared with modern genera of blue green algae. These fossil assemblages are unusual in that they are found in dolomitic limestones and not in chert.

The banded iron formation Gunflint Chert assemblage^{1,2} is associated with stromatolites and is remarkably varied and modern in appearance. Walter¹³ has suggested that these may represent fossilised fumarolic hot springs deposits and that they may have been preserved in primary silica resulting from volcanic activity. The microfossils from other banded iron formations, in Australia for example, do not however, resemble the Gunflint organisms at all, and indeed are much more similar to the spheres from the much older Onverwacht and Fig Tree rocks, both in appearance and in the state of organic metamorphism. The differences may result from original facies variations, or from the very slight age difference between the Australian (2.0×10^9 yr) and the Canadian (1.9×10^9 yr) rocks.

Until fairly recently, there was nearly as large a gap in knowledge between the Gunflint assemblages and the Bitter Springs assemblages described by Schopf^{6,7} as between the Gunflint and the Fig Tree. The Bitter Springs assemblage contains many unicellular and multicellular blue green algae, and has a moderately high proportion (about 25%) of cells interpreted as eukaryotic from the presence of preserved organelles and from the type of cell division observed.

Similar cells, containing membrane-bound contents have since been reported from Beck Springs, California (1.4×10^9 yr old)¹⁴, Belcher Island, Canada (1.8×10^9 yr old)¹⁵ and, most recently, from the Bungle Bungle Dolomite, Western Australia (1.5×10^9 yr old)¹⁶. Thus the origin of the eukaryotes must be regarded as having occurred much earlier than has previously been supposed⁷. The Bungle Bungle Dolomite contains many colonial and multicellular microorganisms like the recently reported assemblage from the Amelia Dolomite of the McArthur Group of Australia's Northern Territory, also 1.6×10^9 yr old^{17,18}. Colonial microorganisms have also been recovered from the HYC ore body of the McArthur Group¹⁹, lending strength to the hypothesis that deposition of some stratiform ore bodies may be related to biogenic activity. This same view has been put forward by Lopukhin²⁰, in a review of Soviet Precambrian microorganisms and their economic importance.

Although there now seems to be some evidence for the presence of eukaryotic organisms as long ago as 1.8×10^9 yr, the assemblages from $1.8-1.3 \times 10^9$ yr interval, although very varied, are dominated by prokaryotes^{16-18, 21}. The cells described by Hoffman²¹ from Belcher Island may well represent primitive 'protoeukaryotes', as indeed may

the cells described from the older Animikie Formation of Ontario²².

New assemblages have been described from the later Precambrian^{23, 24}, and these contain cells that can reasonably be interpreted as eukaryotes thus reinforcing some of Schopf's⁶ earlier views. The enigmatic *Chuarina*, with a diameter of between 2 and 3 mm, has been redescribed by Ford and Breed²⁵, and assigned to the Leiospheridia (Acritarcha). All the recorded occurrences of *Chuarina* are from Precambrian rocks, usually shales, less than 1.0×10^9 yr old. The occurrence of these large plant microfossils seems to have stratigraphic importance. Furthermore, the Leiospheridia can be related to the present day eukaryotic Prasinophyceae.

By the very late Precambrian, complex green algae, similar to *Botryococcus* have been observed in the lower part of the Postpilitic Group from Vrané, near Prague²⁶.

These recent discoveries tend to modify earlier views⁶ that "gradually accelerating early evolutionary development, beginning rather slowly but markedly quickening with the emergence of eukaryotic organisation, seems consistent with the fragmentary evidence currently available". A model of a more gradual, steady, development seems to fit the present evidence much better. Furthermore, the new evidence includes the discovery of microfossils in lithologies other than the facies-restricted black, usually stromatolitic cherts of earlier years, and these will undoubtedly greatly enlarge knowledge of Precambrian microbiotas. M.M.

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Help for the painful phantom limb?

THE phantom limb is a phenomenon that has long aroused curiosity. How is it that one feels a limb that is not there? Yet once one thinks about the anatomy and physiology of the situation, one realises that phantom limbs are to be expected: all the nerves coming from the limb are still there, they still make connections at various levels of the central nervous system and, finally, these connections connect to that part of the brain where afferent nerve impulses evoke sensation. Phantom limbs occur in 95 to 100% of all people who have had a limb or part of a limb amputated.

In that other 5% or less, the limb may still be conjured up by concentrating on it, or by trying to move it.

In most limb amputations, the cut nerves end up in firm swellings called neuromata which consist of whorls of outgrowing nerve fibres surrounded and intertwined by connective tissue. One assumes that these nerve fibres are capable of working and that they frequently fire off impulses to the central nervous system. Not only is this a likely assumption, but it is reinforced by the effect of blocking the cut nerves of the neuroma with a local anaesthetic. When these nerve fibres are blocked in this way, there is a great reduction of sensation or a total loss of the sensation of the phantom limb. It is apparent that the sensation was due to the nerve fibres within the neuroma continually firing off impulses.

A few people who have had parts of their bodies amputated, experience constant pain in the stump or in the phantom, or both. In such patients, blocking the neuromata with local anaesthetics almost always temporarily stops the pain. Many suggestions have been proffered as to why some of the patients have pain and others do not. It could be caused by a difference in the make-up of the neuromata, but this is an unsatisfactory suggestion because no differences in the histological appearances between pain producing and painless neuromata have been found.

Wall and Gutnick (see this issue of *Nature*) have now induced neuromata in the cut sciatic nerves of rats and have recorded from the posterior rootlets entering the spinal cord. As was expected, they found that the nerve fibres of these neuromata were continually active. Their discharge of impulses could be increased by pressure or tapping on the neuromata and it could be stopped by anaesthetising the neuromata with local anaesthetic solution—all similar to the situation in man.

A more interesting finding was the effect of electrical stimulation of the chronically divided nerve. The nerve on which the neuroma had been induced was divided so that it no longer reached the spinal cord. Electrodes were then placed on the nerve and it was stimulated rapidly for a few seconds. This electrical excitation caused a marked reduction in the nerve's spontaneous activity, which lasted for up to an hour. This finding may suggest that if the nerve fibres can be induced to fire off maximally, they may stop firing spontaneously for a prolonged period. A similar mechanism could account for the effectiveness of one of the treatments for painful neuromata. In 1881, Granville (*Lancet*, i, 286) introduced percussion of the neuroma as a treatment for the chronic pain. This treatment was largely forgotten, revived for a short time during the 1914–18 war, forgotten again, and re-introduced by Russell (*Br. med. J.*, **1**, 1024; 1949) the results being reported by Russell and Spalding in 1956 (*Br. med. J.*, **2**, 68). Sometimes when the patient first hits his neuroma with a mallet and peg, the pain is increased; but the neuroma soon becomes numbed and the pain may then stay away for hours.

During the past few years the transcutaneous electric stimulation of nerves has been introduced for many painful conditions, including painful amputation stumps and painful phantom limbs. This treatment was based on the gate-control theory of pain of Melzack and Wall (*Science*, **150**, 971; 1965). The theory states that whether or not an input to the spinal cord finally causes pain depends on the amount of input arriving in large myelinated afferent fibres on the one hand and small myelinated and non-myelinated afferent fibres on the other. Both groups of fibres are said to affect the small neurones of the substantia gelatinosa, a part of the posterior horn very near the zone of entrance of the afferent fibres. The large fibres excite these neurones and the small fibres inhibit them. These substantia gelatinosa neurones are inhibitory. And so, when these neurones are themselves inhibited, their inhibitory action on the afferent fibres will not be exerted; a massive afferent input will enter

the spinal cord, and pain will result. When these neurones are excited, they exert presynaptic inhibition on afferent fibres, and the input to the spinal cord will be moderate; pain will not occur. Excitation of the large afferent fibres thus reduces the input to the spinal cord and is said to close the gate.

Wall and Gutnick now suggest an alternative way in which electric stimulation might act. It could do so preventing nerve impulses being fired off spontaneously, and, as it is this firing that causes both the pain and the phantom sensation, both would be stopped by electric stimulation. The mechanism by which antidromic stimulation stops impulse generation after stimulation has stopped its unknown. Wall and Gutnick suggest that the excitability of small nerve fibres within the neuroma is altered, by electric stimulation because these endings are abnormal and are not behaving in the same way as do normal nerve fibres; and they bring forward some evidence to show that this is so.

This is probably the first physiological investigation to have been carried out on a traumatic neuroma induced in an animal. It seems as if it has already thrown light on how certain forms of therapy may work; and it suggests that a similar investigation could be carried out in man, now that it is possible to record from human nerves by means of electrodes introduced into the peripheral nerves. P.W.N.

Unpredictability of comets

from a Correspondent

COMET Kohoutek disappointed many people over Christmas by being considerably fainter than predicted. Most of the blame for this disappointment can be laid at the feet of the media-men who seem to hanker after superlatives such as brightest, longest, most spectacular and are not interested in run of the mill comets and the qualified predictions of astronomers. Comets themselves, however, must also carry some of the blame as they are notoriously unpredictable. This cometary fickleness has been stressed in an article by Jacchia, who works at the Center for Astrophysics, Harvard Observatory and Smithsonian Astrophysical Observatory (*Sky Telesc.*, 47, 216; 1974).

The brightness J of a comet can be represented by the formula $J = J_0 \Delta^{-2} r^{-n}$ where J_0 is a constant, Δ is the distance between the comet and the Earth, r is the distance between the comet and the Sun, and n , the power of r , is a quantity that varies from comet to comet and, in certain cases, as a function of r . In an extensive study by Bobrov-

nikoff the mean value of n was found to be 3.3. This indicates that cometary luminosity is produced by two mechanisms; first, the reflection of sunlight from the small dust particles in the coma (if this was the only source n would be 2) and, second, by direct emission from gas molecules, thus producing band spectra. The luminosity increases as the comet approaches the Sun because of the increase in the incident radiation, coma size, gas and dust production and molecular excitation. Increases in luminosity and therefore brightness, however, do not always occur and n has been known to take values that range from -1.77 to +11.40. This variability in the power of r is indicative of the chance one takes when predicting cometary brightness. Jacchia gives as one telling example the case of comet Westphal which was observed as an 8th magnitude object on September 26, 1913. Perihelion passage was due on November 26 but instead of brightening the comet gradually faded being 13 mag at the end of October and 17 mag on November 22, 4 days short of perihelion, when it had been expected to be at its brightest.

Jacchia compares Kohoutek with comet Halley's apparition in 1910. Halley performed excellently having an n of 5 and also brightening in the post-perihelion phase. Kohoutek started well with an n of 4.0 shortly after discovery (justifying the early optimistic predictions) but n dropped to 3.0 in early November 1973 and 2.2 around December 20. At perihelion the comet shed more matter than expected and was easily photographed by the Skylab astronauts, but after perihelion the comet followed a trend one magnitude fainter than it did at the corresponding distance on the way to the Sun—exactly opposite to the behaviour of Halley's comet.

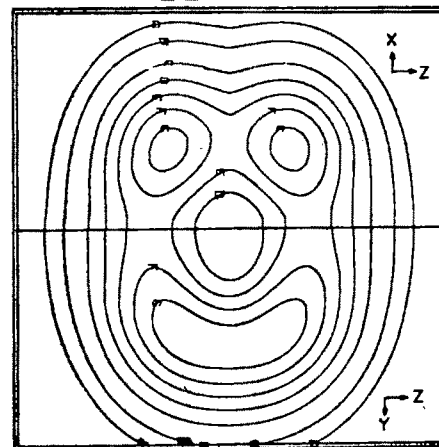
So comets seem to have two records in astronomy. Not only do they have the greatest range of brightness of all celestial objects, the great comet of 1882 being seen in full daylight 4 degrees away from the Sun and comet Wolf 1 being observed in 1942 when it had a magnitude of 19.3, but they also seem to be the most unpredictable: comet Kohoutek unfortunately lived up to this latter reputation.

Man misled by man—on ice

from our Geomagnetism Correspondent

THERE is a standing joke among palaeomagnetists about the man who spent several weeks collecting beautiful samples of baked red laterite. When he returned to the laboratory one of his more experienced colleagues coolly

Happy nuclei



Hartree-Fock calculations of nuclear matter have now advanced to the stage where they can reveal quite detailed and unexpected features of nuclear structure. This illustration shows the contour plot of the density of sulphur-32 obtained by Curry and Sprung (*Nucl. Phys.*, A216, 125; 1973).

informed him that he had drilled the brick foundations of a long-demolished farmhouse. Each branch of the earth sciences has its own variant of this story, but for glaciologists the joke seems to have come uncomfortably to life. As the recent experience of two North American scientists demonstrates, it is easy to be misled by one's ancestors even in the most unlikely of places.

Last year, Berkland and Raymond (*Science*, 181, 651; 1973) reported that they had found glacial polish, grooves and striations at a height of 1,370 m on Grandfather Mountain in North Carolina. The longest of the grooves were about 1 m long, 15 cm wide and 5 cm deep, and the 60 or so recognised examples gradually changed their trend from S80°E to S40°E from the southern to the northern ends of the exposure. This variation, Berkland and Raymond argued, indicated "two lobes of ice which formerly coalesced at the site of the outcrop and preserved it as a bastion". They then went on to calculate that the ice had maximum and minimum thicknesses of 300 m and 100 m, respectively, in the cirque containing the outcrops investigated. It is true that no moraines were observed at the cirque outlet, but this was considered neither unusual nor significant.

What was considered significant was that Grandfather Mountain lies at a latitude of about 36°N, whereas alpine glaciation in the eastern United States had not previously been reported south of the Catskill Mountains in New York state (about 42°N) and the Laurentide ice sheet ended in New Jersey and Pennsylvania between 40° and 41° N. In other words the new result suggested that Pleistocene glaciation had extended some 890 km further south in the

Appalachians than had previously been supposed. This, in turn, raised questions about other evidence which had hitherto been interpreted differently. Striated boulders and cobbles, for example, are quite abundant locally in Pleistocene river gravels of North Carolina and Tennessee and are indistinguishable from glacially striated clasts. Wentworth (*Bull. geol. Soc. Amer.*, **39**, 941; 1928) suggested the possibility of a glacial origin for these more than 40 years ago, although both he and later workers have preferred to ascribe their markings to river ice. The new discovery made by Berkland and Raymond, if substantiated, suggested that the question of glaciation in the southern Appalachians would need to be re-examined.

Unfortunately, the discovery has not been substantiated; that is to say, although there is no doubt that the grooves exist, their origin is certainly not glacial. McKeon (*Science*, **184**, 88; 1974) now reports that he has examined the grooves and has found, among other things, ferric stains in the bottom of some of them, polish on groove bottoms but not on intergroove surfaces, the absence of grooves in shallow lows of the outcrop surface, and the presence of at least one groove continuing part of the way down the outcrop's vertical face. These characteristics, he argues, suggest that the grooves are not glacial but man-made. More specifically, he suggests that the grooves are a product of stone quarrying, logging, or survey activities during the past two centuries—a suggestion strongly supported by the discovery of weathered wire cables downslope from the outcrop. McKeon also uses data from a weather station on Grandfather Mountain to show that glaciation at an elevation of 1,370 m would imply a July glacial age temperature about 18° C lower than the present July temperature—a decrease three times greater than that usually acknowledged for the ancient glacial areas.

Hack and Newell (*Science*, **184**, 89; 1974) have also visited the outcrop concerned and confirm the characteristics described by McKeon, adding that the grooves "in no way resemble the polished, striated, and grooved outcrops [they] have seen in glaciated areas". They also found the rusty steel cables, but were more enterprising than McKeon in seeking out a retired lumberman who had taken part in the last logging in the area during the 1930s. This gentleman described precisely how the grooves were formed by the operation of a steam-powered cable system, adding that similar grooves could be found "on rocks all through the woods". In short, it is clear that the grooves found by Berkland and Raymond are of more interest to industrial archaeologists than to glaciologists.

To their credit, Berkland and Raymond (*Science*, **184**, 89; 1974) openly admit that they were mistaken about the origin of the grooves, at the same time describing at length how they came to be misled. On the other hand, they disagree strongly with Hack and Newell's statement that "no need exists for philosophical discussion of the possibility of alpine glaciation" in the southern Appalachians. On the contrary, they assert that there is a wealth of physical, biological and palaeoclimatic evidence to support such glaciation, and promise that Berkland will discuss this more fully in a later publication. It is clear that, in spite of the set-back of the misinterpreted grooves, the issue of southern Appalachian glaciation is still far from dead.

Palindromes in eukaryotic DNA

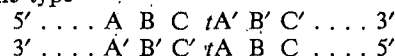
from a Correspondent

GONE are the days when genomes were described solely in terms of the abundance of different repetition frequency classes in eukaryotic DNA sequences. Nowadays it is fashionable to write of the interspersion of repeated and non-repeated sequences and to formulate models of the genome based on the lengths of repeated and non-repeated elements and the extent of intermingling. Good evidence has been provided in *Xenopus* (Davidson *et al.*, *J. molec. Biol.*, **77**, 1; 1973), sea urchins (Graham *et al.*, *Cell*, **1**, 127; 1974), *Drosophila* (Wu *et al.*, *J. molec. Biol.*, **64**, 211; 1972; Lee and Thomas, *ibid.*, **77**, 25; 1973, *Necturus* and mouse (Pieritz and Thomas, *J. molec. Biol.*, **77**, 57; 1973) for such a model. Most of this work, however, has dealt specifically with the intermingling of intermediate repetitive with non-repetitive sequences and not with the fast renaturing, highly repetitive, low complexity DNA, such as mouse satellite DNA, which is thought not to contain any single copy elements over large stretches. The integration of DNA sequences of this type, known to be predominantly in centromeric DNA by *in situ* hybridisation, may be in large blocks alternating with sequences of greater complexity. Evidence in favour of this idea has come from equilibrium caesium chloride density gradient studies on the integration of low complexity satellite DNA sequences in the *Drosophila* genome (Kram *et al.*, *J. molec. Biol.*, **64**, 103; 1972).

There is a fourth fraction of eukaryotic DNA which renatures spontaneously at zero time even at very low DNA concentrations and which has received little attention until recently. Davidson *et al.*, in their paper on interspersion of repetitive and non-repetitive sequences in *Xenopus* also

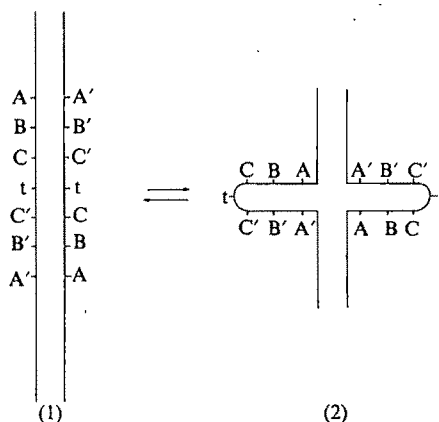
found that this zero time renaturation increases with fragment size and suggest that there is a relatively small fraction of single stranded DNA molecules containing tandem self complementary sequences capable of forming intra-strand duplexes.

Wilson and Thomas (*J. molec. Biol.*, **84**, 115; 1974) now provide further evidence for the existence in eukaryotic DNA of inverted repeated sequences of the type



called palindromes by analogy with words and phrases which read the same backwards as forwards. Denatured palindromes would be expected to renature by an intramolecular reaction to form hairpin loops. The rate of hairpin formation is predicted to be very swift, depending on the number of nucleotides (*t*) separating the inversion, and should proceed at very low DNA concentrations since complementary sequences will not be diluted.

What Wilson and Thomas have done is to use hydroxyapatite, which binds double stranded but not single stranded DNA at low salt concentrations, to prepare spontaneously renaturing DNA from a variety of sources (HeLa cells, mouse L. cells, *Triturus* liver cells, and *Drosophila* flies). They show that zero time binding is independent of DNA concentration and occurs at C_0t values (10^{-5}) of the same order as the $C_0t_{1/2}$ for the reaction between poly(dA) and poly(dT). The only variable which seems to influence the amount of zero time binding is the molecular weight of the original DNA. It increases linearly with molecular weight except in *Triturus* where a plateau is reached with 60-70% DNA bound at molecular weights greater than 10^7 daltons. In HeLa DNA the maximum amount bound increased up to 80% at a molecular weight of 6×10^7 daltons.



(1) Linear palindrome; (2) cruciform palindrome (after Wilson and Thomas).

An alternative explanation that zero time binding is due to the presence of cross linkers in DNA was eliminated by a study of artificially (mitomycin) cross-

linked HeLa DNA. Although a large fraction of the DNA is made to renature spontaneously by treatment with mitomycin, electron microscopy of samples prepared in 90% formamide show a large proportion of X and Y forms, whereas untreated zero time bound DNA shows only linear molecules as predicted.

Observation by electron microscopy of spontaneously renaturing DNA after treatment with a single strand specific nuclease allows measurement of the lengths of the inverted segments. Such measurements show duplex lengths of 300 to 1,200 nucleotide pairs with a very few as long as 6,000. If zero time binding is caused by inverted sequences forming hairpins, then treatment with single strand nuclease, after prior removal of single strand ends, followed by denaturation would be expected to reduce the average molecular weight to half by digestion of the unpaired terminal loop t and also to reduce zero time binding. That neither of these predictions is fulfilled suggests that the terminal loops are resistant to nuclease probably because they are very short. Electron microscopy confirms this since no terminal loops are visible. The thermal stability of HeLa palindromes ($T_m 81.9^\circ\text{C}$) suggests fidelity of base pairing and consequently low levels of base substitution in the inverted segments. Moreover, base composition analysis (43% G+C) of HeLa hairpins shows that they have the same overall composition as total HeLa DNA.

Wilson and Thomas were able to estimate the distances separating adjacent palindromes by measuring molecules in electron micrographs and found that they are located in clusters of two to four. Individual palindromes are separated by predominantly $0.7\ \mu\text{m}$ although some are $8.5\ \mu\text{m}$ apart, but the clusters of palindromes are more sparsely distributed ($40\ \mu\text{m}$). The average spacing of hairpins can also be estimated from the slope of the curve of amount of spontaneously renaturing DNA against fragment size and shows that in HeLa cells palindromes occur every $13.5\ \mu\text{m}$ on average and they are more sparsely located in mouse L cells ($62\ \mu\text{m}$) and *Drosophila* ($75\ \mu\text{m}$). Finally, Wilson and Thomas produce evidence to suggest that the sequences flanking palindromes in mouse L cells are not a random sample of all mouse DNA sequences but are selected. When ^3H -labelled zero time bound DNA is hybridised in the presence of ^{32}P -labelled non-bindable DNA to filters containing unlabelled zero time bound DNA in conditions in which the palindromes have renatured before the reaction, observation of the renaturation of adjacent sequences is possible. Zero time bound DNA preferentially hybridises to ^3H -labelled zero time bound

DNA and not to the ^{32}P -labelled non-bindable fraction. It seems therefore that palindromes are located non randomly in the genome.

Although the evidence for the existence of palindromes is quite good, there is little idea about their functions. Theoretically a palindrome in native DNA may exist in two forms—the normal linear double helix or a 'cruciform' in which the strands have opened and formed two hairpins. A search for 'cruciform' structures in native DNA revealed nothing, however, and thus if they exist they must occur transiently or be stabilised by chromosomal proteins. One clue to their function may be gleaned from the observations on recognition sites in some nucleic acid-protein interactions; for instance, restriction enzymes and the *lac* repressor recognise relatively short palindromes. Furthermore, hairpin loops exist in transfer RNA, ribosomal RNA and perhaps messenger RNA and heterogeneous nuclear RNA and may be responsible for some of the specificity of interaction of these RNA molecules with the appropriate proteins. It is clear that as usual in science description of a phenomenon has outstripped analysis of its physiological importance.

Model interneurone in a locust

from our *Insect Physiology Correspondent*

ONE of the best known of all interneurons is the 'descending contralateral movement detector' of the locust *Schistocerca gregaria*, which is located in the brain and responds to movement in the visual field of the eye on the opposite side of the head. It is particularly responsive to small dark objects the abrupt movement of which evokes a strong brief excitation and a

more prolonged inhibition, associated with the rapid jump of the locust, mediated by motor neurones in the metathorax. O'Shea, Rowell and Williams (*J. exp. Biol.*, **60**, 1; 1974) now provide an excellent example of the way in which modern neuroanatomical techniques can be applied to the insect central nervous system in order to obtain visual preparations and intracellular recordings of neurones known previously only from extracellular records.

The position of the axon was determined by splitting the axon bundles and checking the locations of the well known response characteristics of the axon under study by extracellular recordings. Probing with microelectrodes was then continued and when the unit was penetrated and its identity confirmed by its response characteristics, the axon was marked by injection of cobalt ions through the recording electrode. Iontophoresis of cobalt ions into the axons isolated by cord splitting, followed by application of ammonium sulphide, leads to the visualisation of the axons and their branches in whole cleared ganglia.

The same procedure of microelectrode penetration of the various brain neurones until the characteristic response of the descending contralateral axon was evoked, followed by marking of the cell body by electrophoretic injection led, in favourable preparations, to the filling of the entire neurone and thus to confirmation of the results of iontophoresis of the descending axon from its lower end, and to the demonstration of its thoracic connections.

The cell body of this model interneurone is the largest in the brain, $45\text{--}50\ \mu\text{m}$ in diameter. Earlier electrophysiological studies by Burrows and Rowell had shown that it makes

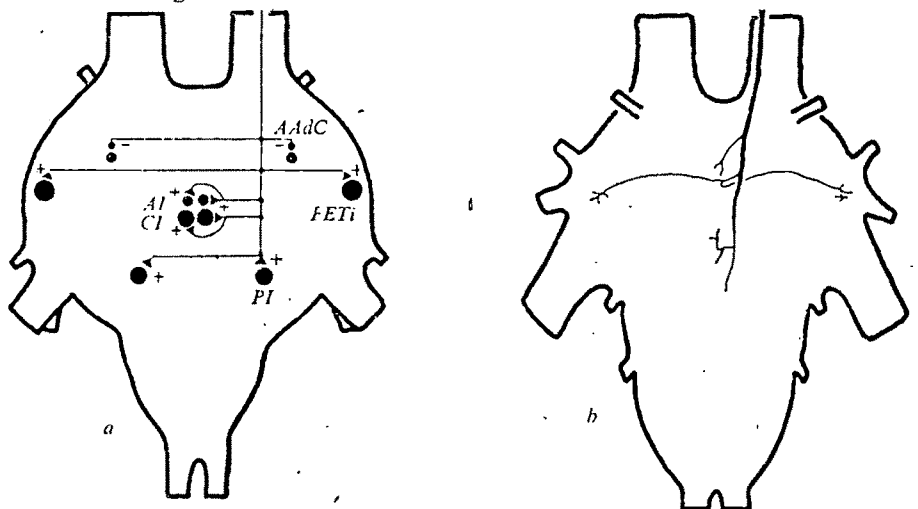


Fig. 1 a, Distribution of the descending contralateral movement detector neurone in the methothoracic ganglion of the locust as inferred from electrophysiological recordings from motor neurones by Burrows and Rowell. The motor neurones are labelled: AAdC, anterior coxal adductor; FETi, fast extensor tibiae; AI, anterior inhibitory flexor tibiae; PI, posterior inhibitory flexor tibiae; CI, common inhibitory. b, The distribution as visualised by cobalt injection.

bilateral connections with various motor neurones in the metathorax (Fig. 1a) ensuring rapid extension of the tibia, with inhibition of the antagonistic flexor muscles and of other systems. The distribution of the axon in the metathorax as revealed in the paper under review (Fig. 1b) shows striking agreement with the conclusions reached by electrophysiological methods alone. There are no major branches to be seen in the metathoracic ganglia which cannot be accounted for by the known connections with motor-neurones involved in the jump. It seems likely that this is the most important function of the descending movement detector neurones. There are also restricted connections with neurones in the prothorax and mesothorax, whose role in the jump are at present unknown. There are considerable individual differences in the detailed arrangements of these neurones. Assuming, as the authors do, that these differences are genetically determined, they may well form the basis of a behaviour variance, in the population, subject to natural selection.

O'Shea *et al.* conclude by pointing out that by using the methods of iontophoresis of cobalt ions, neural anatomists could uncover rich new fields for physiological investigation.

Mapping of poliovirus receptor

from our
Molecular Genetics Correspondent

POLIOVIRUS provides one of those interesting situations in which the susceptibility of a cell to a virus may depend upon its possession of the appropriate receptor. Human cells possess the receptor and can therefore be killed by the virus; whereas rodent cells usually cannot be infected. But in human-rodent hybrid cells, possession of a human gene for the poliovirus receptor is sufficient to enable the virus nucleic acid to enter the cell and, once this first step has been taken, the virus can then multiply without the mediation of any further human gene products, the rodent genetic apparatus being sufficient for its needs. It should therefore in principle be possible to identify the human chromosome(s) carrying the gene(s) concerned with poliovirus reception by making use of human-rodent hybrids which differ in their complement of human chromosomes; when some of the human chromosomes have been shed after hybridisation, loss of the appropriate human chromosome(s) should mean that the hybrids also lose their susceptibility to poliovirus. By performing such experiments, Miller *et al.* report in the April issue

of *Cell* (1, 167; 1974) that a single human chromosome is responsible for conferring upon cells the ability to accept poliovirus.

These experiments have succeeded where earlier attempts to gain this information failed because of two recent developments; hybrid lines are now available with a high degree of homogeneity of human chromosomes (necessary to establish correlations with poliovirus sensitivity), and banding techniques can be used to identify these chromosomes from the others of the set. Virus-resistant and virus-sensitive human-mouse hybrid cells all proved to possess about the same number of human and mouse chromosomes, excluding the possibility that changes in the ratios of two genomes might be relevant in establishing susceptibility to the virus (a mechanism important in polyoma susceptibility, or adenovirus multiplication).

The chromosomes of several poliovirus resistant and sensitive lines were examined by both fluorescent Q banding, which produces characteristic striations of each human and mouse chromosome, and also by C banding, which stains principally the centrometric heterochromatin. Cell lines resistant to poliovirus lacked human chromosome 19, whereas lines sensitive to infection possessed this chromosome. This implies that chromosome 19 carries all the information necessary for acceptance of poliovirus by a human cell. This does not prove, of course, that only a single gene codes for the receptor protein; but this is the most likely interpretation of these results since the genes carried on several chromosomes were responsible, the loss of each of these chromosomes might produce the receptor-deficient phenotype. The idea that more than one gene might be involved, but that all may be located on chromosome 19, may be excluded in future experiments.

Interference in heavy ion inelastic scattering

from our
Nuclear Theory Correspondent

ONE OF THE most interesting features of heavy ion interactions is the interference effects between the different processes that can take place when the interacting ions differ by only a few nucleons. For example, if ^{16}O is scattered by ^{17}O , the neutron transfer reaction from ^{17}O to the ground state of ^{16}O gives the same emerging particles as the simple elastic scattering and so is indistinguishable from it. The corresponding amplitudes interfere quantum mechanically and if they are of comparable

New MRC unit

THE Medical Research Council set up on April 1 a Mammalian Development Unit in University College, London. Its head is Dr Anne McLaren who previously worked in the Agricultural Research Council Unit of Animal Genetics in Edinburgh. She will be assisted initially by three other senior scientists two of whom are Dr M. H. L. Snow, also from the ARC Unit of Animal Genetics, and Dr D. G. Whittingham who is at present attached to the Physiological Laboratory in Cambridge.

The unit will study the growth and differentiation of the mammalian embryo, concentrating on the expression of genes in the early post-implantation embryo. Some of this work will be done in collaboration with Professor H. Harris. Dr McLaren will continue to use mice as her main experimental animals.

Dr Whittingham plans to extend his work on the low temperature storage of mouse embryos in collaboration with Dr Mary Lyon. The practical application of this work is in livestock breeding but many experiments, such as those on the mutagenic effects of irradiation on the stored embryos, can only be done with an animal whose genetics are well understood.

The new unit is being housed in the former home of Professor H. Grüneberg's Experimental Genetics Unit which was disbanded in 1972. Its budget is £175,000 for five years, unless it expands quickly enough to need more.

magnitude this is evident in the observed elastic scattering cross sections.

Interference phenomena are sensitive to the relative amplitudes and phases of the contributing process so they can provide a detailed check of the models used to calculate them, in this case of the distorted wave theory of the interaction and the optical potentials used, as well as of the spectroscopic factors used to obtain the transfer amplitude. Many studies of elastic scattering of similar ions are being made with these aims in mind.

A group at the Max Planck Institute for Nuclear Physics in Heidelberg (Gelbke, Baur, Bock, Braun-Munzinger, Grochulski, Harvey and Stock, *Nucl. Phys.*, A219, 253; 1974) has recently obtained evidence for the occurrence of similar interference phenomena in the inelastic scattering of similar heavy ions. They bombarded ^{16}O with ^{17}O ions with energies ranging from 22 to 32 MeV and measured the cross section for the

reaction leaving a ^{17}O ion in its $\frac{1}{2}^+$ state at 0.871 MeV.

This process can take place in at least two ways. First, the energy of the interaction can simply promote the odd neutron in ^{17}O from its ground state orbit to the one corresponding to the 0.871-MeV state; this is the normal inelastic scattering mechanism. But an experimentally indistinguishable result is obtained if the odd neutron in the incident ^{17}O ion is transferred to the orbit in the target ^{16}O that leaves the resulting ^{17}O nucleus in its 0.851-MeV state. This process gives a cross section that is peaked in the backward direction whereas the former process gives one peaked in the forward direction. At the energies of this experiment both amplitudes have broad angular peaks and similar absolute magnitudes, so significant interference effects can occur.

These cross sections were calculated using the distorted wave theory and the results obtained for the dominant transfer inelastic process above are shown in Fig. 1. The overall behaviour of the cross sections is given quite well but close examination shows fine structure in the data that is not reproduced theoretically. Additional calculations were then made including the contribution from the direct inelastic scattering process, and the results (Fig. 2) show that the fine structure is at least qualitatively reproduced. A perfect fit is not to be expected because higher order processes are not taken into account explicitly in this work. Some implicit account was taken of them by multiplying the direct inelastic scattering amplitude by a factor of 1.81 and this gives a much improved fit.

This work provides convincing evidence for the presence of interference

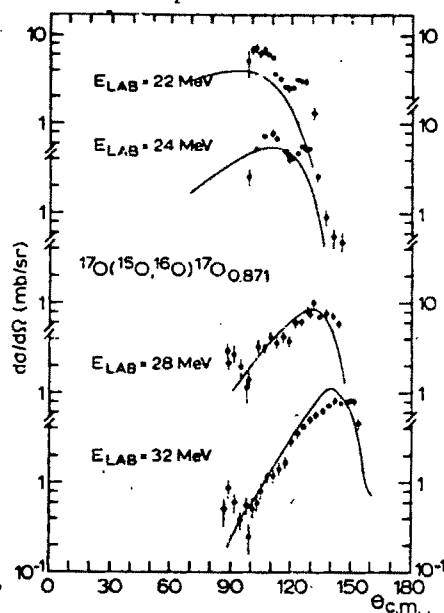


Fig. 1 Differential cross sections for the inelastic scattering of ^{16}O by ^{17}O compared with distorted wave calculations of the one-neutron transfer process.

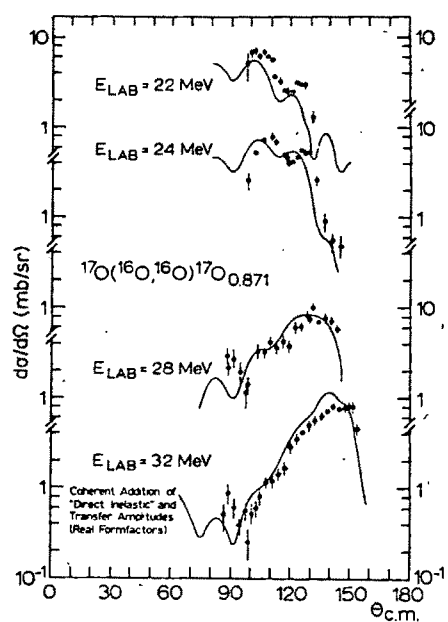


Fig. 2 Differential cross sections for the inelastic scattering of ^{16}O by ^{17}O compared with distorted wave calculations of the coherent addition of the direct inelastic and inelastic transfer process.

effects in the inelastic scattering of heavy ions and is likely to develop into a useful spectroscopic tool.

Gene isolation and HnRNA

from a Correspondent

HnRNA, an abbreviation for the more cumbersome 'heterogeneous nuclear RNA', has been a difficult, yet intriguing, class of RNA molecules to study. Although it was discovered in 1961, clear and formal evidence that HnRNA is a high molecular weight intermediate, or precursor, in the biosynthesis of messenger RNA has been discovered only recently. The turning point came (as J. Darnell of Columbia University, New York, explained at a symposium of the nucleotide group of the British Chemical and Biochemical Societies held in Glasgow on April 1 and 2) when it was found that both HnRNA and mRNA have the common and distinctive chemical feature of an extensive polyadenylic acid region at one end of the molecule.

These studies were made initially on the complex mixtures of both messenger RNA and of HnRNA present in a widely used human tumour cell line of epithelial origin (HeLa cells). The same type of precursor-product relationship has, however, been convincingly shown by both R. Williamson (Beatson Institute, Glasgow) and K. Scherrer (Institute for Cancer Research, Lausanne) for the biosynthesis of the specific globin messenger RNAs in the specialised red blood forming cells. Scherrer emphasised the complexity of the maturation

process of the highest molecular weight HnRNA to the end product globin mRNA even in this well defined system. The biosynthesis of the heavy chain immunoglobulin mRNAs (A. R. Williamson, University of Glasgow) may be simpler as only two well defined sizes of HnRNA occur.

In the maturation of HnRNA at least 90% of the molecule is degraded in the nucleus. Both Darnell and R. H. Burdon (University of Glasgow) described uridylyte-rich regions in this part of HnRNA. Furthermore, extensively repeated helical regions occur (Darnell).

The control of the synthesis of HnRNA is an area where it is easier to suggest mechanisms than to verify whether these mechanisms actually operate. R. S. Gilmour (Beatson Institute, Glasgow), however, is studying the induction of haemoglobin synthesis in chromatin (thus studying genes which are actively capable of being expressed) in a 'Friend' leukaemic cell, has isolated a mutant with more globin mRNA. This may be a 'transcriptional' mutant with an increased rate of synthesis of HnRNA. By contrast, the absence of α -globin synthesis in a still-born child suffering from *Hydrops foetalis* (an extreme form of α thalassaemia) is caused by the absence of the α -globin gene rather than by any fault in synthesis of mRNA (R. Williamson).

Two speakers, D. D. Brown (Carnegie Institute, Baltimore) and M. L. Birnstiel (University of Zurich) described the isolation and some properties of specific animal genes. Brown has made an extensive study of the reiterated genes coding for 5S ribosomal RNA in the South African toad (*Xenopus laevis*) and he presented new evidence of the size and sequence heterogeneity of the 'spacer' regions which alternate with the coding regions in this extensively repeated gene. Furthermore, recent sequence studies on the spacer have shown that it consists in part of many short identical or nearly identical sequences in tandem, the average repeat length being sixteen bases.

The importance of the radioactive sequence methodology was emphasised by F. Sanger (MRC Laboratory of Molecular Biology, Cambridge) when he described his recent work on sequencing DNA by radioactive methods. DNA may be labelled either 'directly' or by 'copying' methods. The first approach was used to derive two 50-long sequences in the bacteriophage ΦX ; using the second approach he described the methods for extending a primer complementary to the single stranded DNA of bacteriophage f1 using DNA polymerase to deduce a sequence of 81 residues. Undoubtedly the methods will be of very general application to many problems in molecular biology.

Photometry of Comet Kohoutek (1973f)

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Extensive photoelectric and infrared photometry of Comet Kohoutek (1973f) shows that its nucleus is 10 to 15 km in diameter and that the comet contains relatively little dust, and provides new information about the nature of cometary dust grains.

We observed Comet Kohoutek (1973f) photometrically from 0.3 to 30 μm and at heliocentric distances from 0.37 to 1.9 AU. Short of 1.5 μm , the flux consists of emission lines and sunlight scattered by cometary dust; the observations in this spectral region show that the nucleus is no larger than those of a number of other recent comets. Thermal re-emission

from the dust is dominant beyond 2 μm ; our data show that Comet Kohoutek contains relatively little dust. These two results explain why the comet was much less spectacular visually than had been expected. The data reported here provide the first large, homogeneous set of photoelectric and infrared photometry obtained for any comet, and they provide new information on the nature of cometary dust.

By necessity, most of our observations were made at very large zenith angles and are therefore less accurate than normal photometry. Single measurements are probably uncertain by at least 10% and even greater uncertainties apply to observations near 20 μm (because of the large extinction corrections at this wavelength) and to those made with a

TABLE 1 Photoelectric photometry of Comet Kohoutek (1973f)

Time of observation Date	r(AU)	Diaphragms (arc s)						Magnitudes				
		12	31	48	62	112	157	U	B	V	R	I
October 8.5	1.92			x					12.38	11.71		
October 16.5	1.79		x						12.37	11.83		
October 18.5	1.75				x				11.45	10.62		
			x						12.41	11.65		
October 19.5	1.74					x			10.79	10.08		
					x				11.68	10.92		
			x					12.69	12.63	11.85		
October 22.5	1.68		x			x			10.66	9.88		
			x						12.36	11.57	10.88	10.53
October 25.5*	1.63					x			10.21	9.48		
			x						11.95	11.17	10.77	10.40
		x							14.05	13.04		
October 26.5	1.61					x	x		10.06	9.24		
			x						10.31	9.53		
								12.07	12.14	11.53		
October 28.5	1.57	x						14.11	13.97	13.02		
						x	x		9.92	9.28		
			x					9.91	10.17	9.54		
October 31.5	1.52						x	11.95	12.05	11.29	10.68	10.22
					x				9.66	8.98		
						x		9.85	10.31	9.28		
				x					10.80	10.05		
			x						11.07	10.34		
November 1.5	1.50						x	9.00	9.70	8.99	8.83	8.29
					x			10.38	10.78	10.06		
			x					11.59	11.82	11.15	10.63	10.15
November 5.5	1.42	x							13.57	12.87		
		x							13.62	12.71		
					x		x	8.97	9.41	8.71	8.60	8.26
								10.18	10.47	9.73		
November 6.5	1.40		x					11.50	11.64	10.90	10.47	9.98
							x	9.04	9.37	8.71	8.60	8.24
			x					11.56	11.65	10.97	10.52	10.16
		x						13.51	13.37	12.60		
November 11.5	1.30						x	8.60	8.91	8.18		
November 14.5	1.24						x	8.15	8.68	7.92		
					x			9.44	9.80	9.05		
			x					10.70	10.97	10.28	9.90	9.44
		x						12.83	12.77	12.09		
November 16.5	1.20						x	8.06	8.56	7.87	7.94	7.42
			x					10.75	11.01	10.22	9.90	9.35
					x			9.53	9.70	9.00		
November 28.5	0.94						x	7.26	7.67	6.97	7.13	6.43
					x			8.51	8.76	8.01	7.93	7.27
			x					9.78	9.98	9.26	9.06	8.48
December 9.5	0.67						x		6.14	5.76	5.86	5.21
			x					7.92	8.20	7.60	7.45	6.95
		x						9.86	10.11	9.43	9.14	8.92

* Extinction coefficients not well determined.

TABLE 2 Infrared photometry of Comet Kohoutek (1973f)

Time of observation Date		Diaphragms (arc s)			Fluxes (Jy)*									
		13.5	6.8	5.5	2.2 (0.6)	3.6 (0.9)	5.0 (1.0)	8.8 (1.0)	10.3 (1.2)	10.6 (5.0)	11.6 (0.8)	12.6 (1.0)	21 (8)	22.5 (5)
October 7.5				x						1.2	1.2	1.6		
October 8.5				x						1.0	1.0	1.8		
October 16.5				x						1.7			2.8	
October 18.5				x						1.6	1.9		3.6	
October 23.5	x									4.6				
October 24.5	x									4.2				
			x							2.6				
November 1.5	x									5.3				
November 9.5				x			2.3	3.9		5.2	5.5	4.4	7.8	
November 15.5	x									14.8				
				x	0.04		0.43	6.2	10.0	8.0	8.9	8.3	18	25
November 28.5	x						46	56		47	49	36	69	
December 6.5	x					3.6	17	81	120	80	98	85	83	98
			x							52				
December 9.5	x					9.4	27	123	170	165	186	158	140	146
December 13.6	x				4.3	40	100	280	520	360	380	370		
December 18.7				x	11	76	160	320	560	450		340	320	220
December 19.7	x					330				1250				
				x	26	175	360	630	800	670	630	450	550	420

* The measurements are identified by the centre wavelength of the filter in μm , followed by the band width. $1 \text{ Jy} = 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$.

12 arc s beam in the photoelectric region (because of the very diffuse nature of the comet in this spectral range).

Photoelectric photometry was carried out in the *UBVRI* system defined by Johnson^{1,2} (Table 1) and infrared observations were carried out with a number of filters (Tables 2 and 3; the absolute calibration is from Low³). The data are presented graphically in Fig. 1; the infrared data have been normalised to a beam of 13.5 arc s by means of the observed dependence on beam size of the flux at 10 μm . Scans through the nucleus of the comet at 3.6 μm and 10.6 μm (see Fig. 2) show the angular distribution of the flux to be very similar at the two wavelengths and support the use of a single correction to the beam size throughout the infrared.

To interpret these observations, it is important to know the contribution of emission lines to the flux observed in each filter. A spectrum obtained by R. E. White (personal communication) on October 16.5 shows that all the photoelectric photometry near this date should be dominated by the continuum, with the possible exception of the *U* band which may be affected by CN emission near 3,880 Å. The rapid brightening of the emission lines after this date is apparent in the relatively rapid brightening in *U*, *B* and *V*. The *R* and *I* bands should be dominated by the continuum throughout our observations. A spectrum obtained by W. Wisniewski (private communication) on December 10.5 shows that the emission lines near the nucleus are much weaker than the continuum in these two bands. Detailed interpretation of the *UBV* photometry since October must await the availability of more spectroscopic observations; when such data are available, the photometry should make it possible to refer all the measurements to a common region centered on the nucleus.

Swings⁴ has listed a large number of infrared emission lines that might be expected from comets. If these lines were strong, they would be apparent in our photometry; for example, bands of CO, CO⁺, CN, CN⁺ and N₂O should

be included in our measurement at 4.8 μm and few additional lines should be added with the wider bandpass centred at 5.0 μm . The smoothness of the spectrum in this region and between 3 and 4 μm , where many other molecular bands fall, implies that the contamination of the infrared photometry by emission lines is small. Higher spectroscopic resolution near 2.2 μm on Comet Ikeya-Seki (1965f)⁵ and near 2.2 and 3.5 μm on Comet Bennett (1969i)⁶ showed negligible contamination of the broadband photometry at these wavelengths by emission lines. The emission peaks at 10 and 18 μm are also seen in the spectra of many infrared stars and are generally attributed to dust containing silicates. The feature at 10 μm was previously detected in Comet Bennett⁷.

Our photometry allows a revised estimate for the diameter of the nucleus of the comet. The initial value of 50 km was based on photographic measurements and an assumed albedo of 0.25. But reflection from a nucleus of 50 km with an albedo of 0.25 would account for the entire signal observed photoelectrically in October with the beam of 12 arc s. Photographs of the comet at that time show that it had a well developed coma which would account for a substantial part of the flux within this beam; therefore, the diameter of the nucleus must have been overestimated. We have extrapolated our photometry to smaller beams in order to estimate the magnitude of the nucleus toward the end of October. A variety of extrapolation procedures yields a range of $14.5 > m_v > 16$; adopting $m_v \sim 15$ and assuming an albedo of 0.5 (a high albedo has been assumed because of the large ratio of gas to dust in this comet), we arrive at a revised value of 10 to 15 km for the diameter of the nucleus. In other words, the nucleus of Comet Kohoutek (1973) is probably no larger than the nuclei of several recent, bright comets⁸.

For detailed comparison, the infrared spectrum of Comet Bennett (1969i)⁷ is included in Fig. 1. If this spectrum were adjusted downwards in flux by a factor of 30, it would fit smoothly into the progression of spectra for Comet Kohoutek

TABLE 3 Additional infrared photometry on December 19.7

Wavelength	1.6	3.05	3.88	4.8	10.8	17	19	24.5
Bandwidth (μm)	0.3	0.1	0.5	0.6	1.0	2.0	1.0	1.0
Fluxes* (Jy)	10	100	180	290	630	530	550	410

* 5.5 arc s beam

except that the feature at $10\ \mu\text{m}$ and the flux in the region 3 to $5\ \mu\text{m}$ are somewhat stronger in Comet Bennett. Since the dust in the two comets is evidently of roughly similar composition, we can compare the amounts of dust by correcting the fluxes for the differences in field of view and geocentric distance. The result is that Comet Bennett had about 16 times as much dust as Comet Kohoutek.

The original predictions that Comet Kohoutek might have $m_v \sim -8$ at perihelion passage were based on the observations of Comet Bennett, renormalised to allow for a nucleus about two times larger in diameter. Since the amount of matter released by a comet nucleus is proportional to the surface area, the downward revisions in the diameter of the nucleus and in the amount of dust would imply that the visual magnitude of Comet Kohoutek was overestimated by about four magnitudes. This result agrees well with the observed magnitude of Comet Kohoutek and indicates that the corrections we have applied are an adequate explanation for its disappointing performance.

The heliocentric distance of the comet in October considerably exceeds those at which infrared observations of comets were available previously⁵⁻⁹. Our measurements indicate no emission peak at $10\ \mu\text{m}$ at large distances from the Sun. This transition should be confirmed as the comet leaves the Sun; it correlates well with recent calculations that high albedo clathrate grains ejected from a comet nucleus would have long lifetimes until the comet approached to within 1.5 to 2.0 AU of the Sun¹⁰.

O'Dell¹¹ has derived an expression for the albedo of cometary dust grains in terms of the integrated infrared surface brightness and the surface brightness of scattered light in the continuum. Our data are the first which permit an accurate evaluation of this expression; they yield

$$\gamma/(1 - \gamma) = 0.16$$

where γ is the albedo. The mean absorption efficiency of the grains in the visible region is then

$$\epsilon_A = 1.0/[1.0 + 0.16 \alpha(\theta)]$$

where $\alpha(\theta)$ is the ratio of the scattering efficiency of the grains averaged over all angles to the scattering efficiency at the angle of observation, θ . Over the range of θ for our observations, $\alpha(\theta) \gtrsim 1$, so that $\epsilon_A \approx 0.8$.

The infrared observations also provide information about $\epsilon(\lambda)$, the infrared emissivity of the dust grains as a function of wavelength. The temperature of the grains is determined by $\epsilon(\lambda_{\text{max}})/\epsilon_A$ at the wavelengths, λ_{max} , where significant infrared emission occurs. At a given temperature, the spectrum of the grains is controlled by $\epsilon(\lambda)$. Infrared observations at a single heliocentric distance can be fitted by a variety of emissivity laws if appropriate values are assigned to $\epsilon(\lambda_{\text{max}})$. But observations over a large range of heliocentric distance provide much more information about $\epsilon(\lambda)$, since the evolution with changing temperature of any assumed composition and size distribution of grains must be consistent with the infrared spectra.

Major changes in the nature of the dust grains should be reflected in changes in ϵ_A . Starting in November, when there is sufficient spectral coverage in the infrared to evaluate this parameter, it remains approximately the same through the final photoelectric observation on December 9.5. Observations by Ney and Hefele¹² and Ney and Ney¹³, combined with our data, indicate no major changes in ϵ_A at least to 0.23 AU.

If the nature of the dust grains does not vary, observations over a range of heliocentric distance in principle determine a unique $\epsilon(\lambda)$. As the comet moves from 1.3 AU to 0.2 AU, the equilibrium temperature of the dust grains (defined as the temperature they would assume if $\epsilon(\lambda_{\text{max}}) = \epsilon_A$) varies from 250 K to 600 K. Therefore, the grain temperature through this range of heliocentric distance is determined by the emissivity between 4 and $15\ \mu\text{m}$. We have carried out calculations assuming $\epsilon(\lambda) = a \epsilon_A \lambda^b$ over this spectral interval and requiring that the calculated spectra agree with

the observations at 5 and $12.6\ \mu\text{m}$ (two wavelengths chosen to avoid complications from the weak silicate feature at $10\ \mu\text{m}$). We find that $b \leq 0$ gives a poor fit, particularly to the data at 0.23 AU (ref. 13) and also requires $\epsilon(\lambda) > 1$ near $3\ \mu\text{m}$. Much better results are obtained with $1/2 \lesssim b \lesssim 1$; $\epsilon(\lambda)$ then has a value of ~ 0.2 at $10\ \mu\text{m}$. In other words, if the nature of the grains has not varied with changing heliocentric distance between 1.3 and 0.2 AU, their emissivity falls from ~ 0.8 in the visible to a minimum of ~ 0.1 near $5\ \mu\text{m}$ and slowly rises toward longer wavelengths through $20\ \mu\text{m}$. Although we have implicitly assumed that all the dust grains have similar composition, most of these conclusions hold for the average properties of dust made up of several kinds of grains, as long as most of the emission

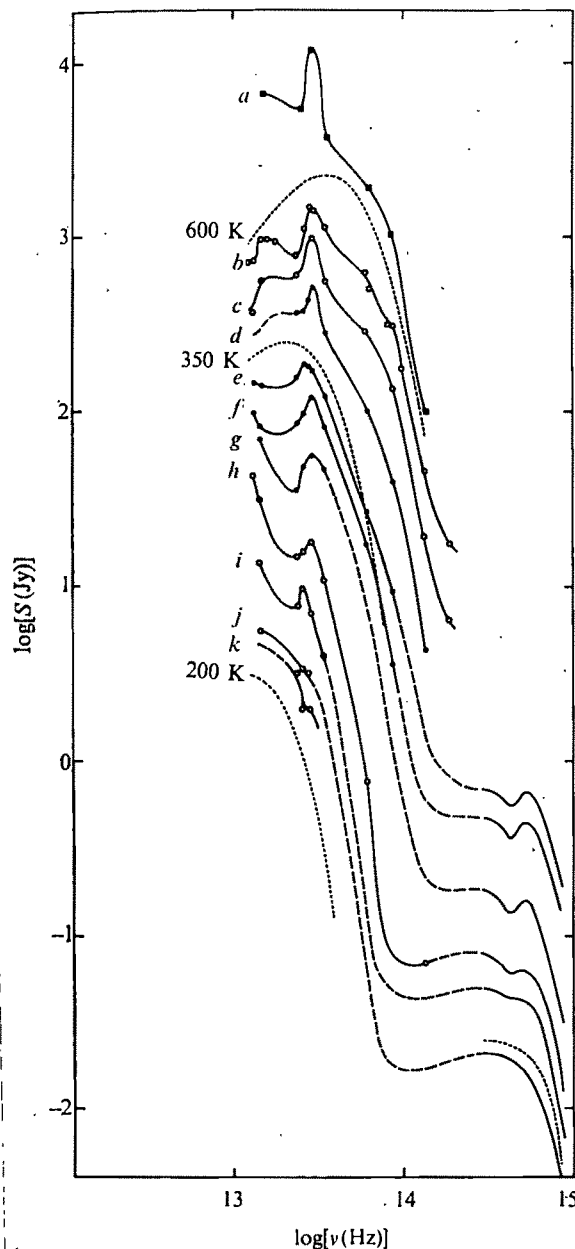


FIG. 1 Photometry of Comet Kohoutek (1973f). The spectra are identified by dates (UT) and heliocentric distances. The spectrum of Comet Bennett (1969i) from Maas *et al.*⁷ is included for comparison. Also shown are a number of blackbody curves in the infrared and a renormalised solar spectrum in the photoelectric region. a, Comet Bennett (0.64 AU); b, December 19.7 (0.37 AU); c, December 18.7 (0.40 AU); d, December 13.6 (0.56 AU); e, December 9.5 (0.67 AU); f, December 6.5 (0.74 AU); g, November 28.5 (0.94 AU); h, November 15.5 (1.22 AU); i, November 9.5 (1.34 AU); j, October 17.5 (1.77 AU); k, October 8.0 (1.95 AU).

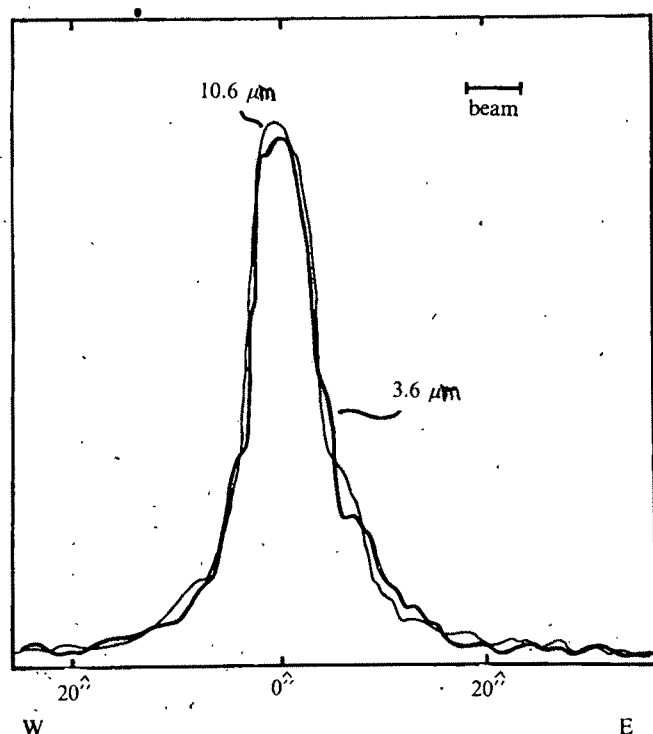


FIG. 2 Scans through the nucleus of Comet Kohoutek (1973f) at 3.6 and 10.6 μm . The observations were made on December 19.7 with a beam of 5.5 arcs.

between 4 and 15 μm is produced by a single type of grain. The scans shown in Fig. 2 imply that a single kind of grain is responsible for the emission at both 3.6 and 10.6 μm , since different spatial distribution would be expected for grains with significantly different properties.

These specific conclusions about the dust grains should

be compared with calculations of the properties of small particles of different compositions, such as those carried out by Krishna Swamy and Donn¹⁴. At present, we do not know of any grain compositions that would have the required properties. Although grains with suitable characteristics may be found, our data favour models in which the emission between 4 and 15 μm is produced by more than one kind of grain or in which the infrared properties of the grains vary between heliocentric distances of 0.2 and 1.3 AU. Models of the first kind must explain the similar spatial distributions of the flux at 3.6 and 10.6 μm , and models of the second kind must be made consistent with the lack of variation observed in the absorption efficiency of the grains in the visible.

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Properties of afferent nerve impulses originating from a neuroma

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Damaged nerves attempt to regenerate. The nerve membrane changes its properties, becomes spontaneously active and may be the source of pain. These impulse generators have unusual properties. They become silent after high frequency activity. This silence may partially explain the effect of counterstimulation as a pain therapy.

WHEN a peripheral nerve is cut, fine unmyelinated sprouts grow out from the central cut ends of the axons. If these sprouts fail to enter the distal part of the cut nerve, they form a tangled mass in the region of the cut. This mass, the neuroma, contains poorly vascularised connective tissue infiltrated with large numbers of the sprouts of the parent axons. The sprouts extend 1-2 mm from the parent axon and they may curve back and enter the connective tissue around the nerve of origin¹. When a neuroma occurs in man

after a peripheral nerve has been cut across and regeneration has failed, it may be a source of pain. Neuromas in man must be the source of nerve impulses and contain mechanoreceptive endings since they are always tender when gently palpated. In some cases, they produce an ongoing barrage of nerve impulses since infiltration of the neuroma with local anaesthetic abolishes pain which these patients refer to the area supplied by the damaged nerve. In spite of the clinical importance and the basic interest of neuromas, physiological studies of them have not been carried out. The physiology of the growing tips of regenerating sensory nerves has been examined² and, unlike normal axons, they were shown to be easily excited by mechanical distortion and by acetylcholine. No mention was made of ongoing activity being generated in the growing tips but it should be remembered that these regenerating fibres are surrounded by their normal surround cells unlike those in a neuroma. Immediately after normal axons are cut across or crushed, a high frequency

injury discharge is generated in all types of nerve fibres but this discharge disappears within seconds and the fibre becomes silent at least for some hours³. The study reported here was designed to investigate what happened some days and weeks after nerve section when a neuroma was established.

An in vivo-in vitro neuroma

Chronic experiments were carried out on 27 adult rats of the Hebrew University strain weighing 250–450 g. Under anaesthesia, the sciatic nerve was exposed and sectioned in the upper leg. A suture was placed around the cut end of the nerve and 5–8 mm of the nerve was drawn into a close fitting medical polyethylene catheter tube. The distal end of the tube was sealed by heat forming a chamber containing the cut end of the nerve and a length of normal vascularised nerve. The nerve chamber was replaced in the leg and the animal was allowed to recover. On examination of the animal the distal musculature was found to be paralysed and the lateral two-thirds of the foot seemed completely anaesthetised. In 6 of the 27 animals, the animal mutilated the anaesthetised part of its foot after recovery periods of 9–14 d. This phenomenon has been reported before⁴ and may suggest that the animals were suffering some form of anaesthesia dolorosa or phantom pain. If this self mutilation occurred, the animals were at once reanaesthetised, examined and killed but the results did not differ from those from non-mutilating animals who were examined 9–40 d after nerve section.

For the terminal study each animal was anaesthetised with urethane, 750 mg/kg⁻¹, paralysed with gallamine and placed under artificial respiration. The nerve chamber was exposed and microscopic inspection through the wall of the tube showed a terminal neuroma occupying the distal third of the tube with the normal nerve surrounded by loose connective tissue running proximally through the tube and out of the open end. Two 28 gauge hypodermic needles were installed in the distal sealed end of the tube for use as stimulating electrodes and as a way to perfuse solutions through the chamber. The opposite intact sciatic nerve was exposed at the same distance from the spinal cord as the neuroma so that control stimulation could be applied to the intact nerve or to acutely sectioned nerve. For recording, the caudal equina was exposed in an extensive lumbar laminectomy so that the dorsal roots of both sides were available for dissection in an oil pool. Dorsal roots were cut close to the spinal cord and small filaments were dissected free and placed on recording electrodes.

Electrical stimulation

For each animal, a compound potential was recorded on a dorsal rootlet following stimulation of the neuroma (Fig. 1a). For comparison, a stimulus was also delivered at a different time to the dorsal root ganglion region of the dorsal root from which recordings were taken. The same was done on the normal side by recording from a cut dorsal rootlet following stimulation of its ganglion and its intact sciatic nerve at the same distance as the neuroma. The earliest waves on the abnormal and normal sides which result from dorsal root ganglion stimulation were identical and represent the arrival of a volley of which the fastest impulses were conducted at 59 m s⁻¹. When the sciatic nerve was stimulated on the normal side, a delayed compound action potential was recorded because of the longer conduction distance. The earliest component of the action potential was produced by impulses whose overall fastest velocity was 54 m s⁻¹. When the neuroma was stimulated, however, a very delayed compound action potential was recorded even though the conduction distance was identical on the two sides. This compound action potential has two waves, the earliest generated by impulses with an overall fastest conduction velocity of 29 m s⁻¹ and the second with velocities up to 15 m s⁻¹. At

a higher stimulus strength, even slower components were recorded. The delay in arrival of impulses is caused by very slow conduction of impulses in the neuroma (Fig. 2a). Here two compound action potentials are shown; the later one is generated by stimulation within the neuroma and the earlier by stimulating the sciatic nerve 5 mm proximal to the tip of the neuroma. The recording was, as before, on a dorsal rootlet. The fastest impulses from the proximal stimulation arrived at 52 m s⁻¹ but the impulses generated

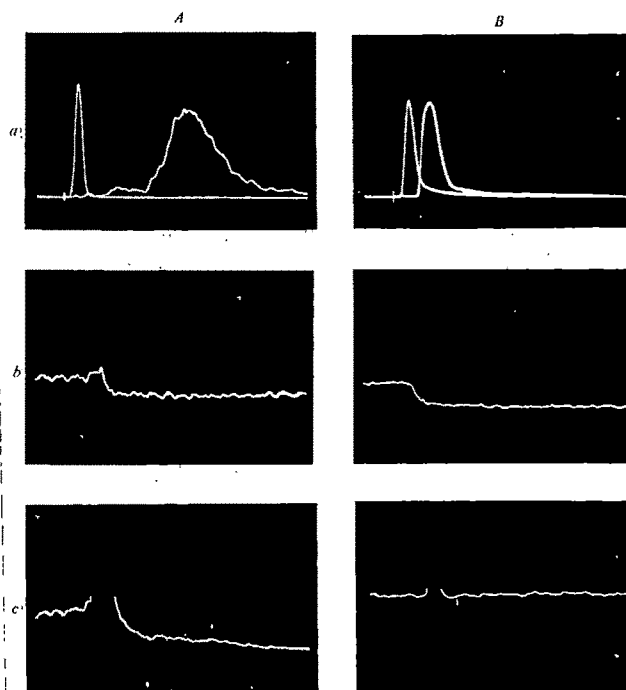


FIG. 1 Recordings from a dorsal rootlet originating from a neuroma (A), and from normal intact sciatic nerve (B). a—Two superimposed sweeps. The early fast wave on the neuroma side and on the normal side were recorded on centrally cut dorsal rootlets following stimulation in the region of the dorsal root ganglion of the recording rootlet, conduction distance 13 mm, fastest velocity 59 m s⁻¹. The slower waves were recorded following stimulation of the normal sciatic at a distance of 45 mm from the recording point and following stimulation of the neuroma also 45 mm from its recording point. The overall fastest velocity from the normal sciatic was 54 m s⁻¹. The velocities for the first components of the two waves originating from the neuroma were 29 and 15 m s⁻¹. Time mark 2 ms.

b—The rate of ongoing activity in a rootlet originating mainly from a neuroma on the left and of ongoing activity in a rootlet originating mainly from an intact sciatic nerve on the right. Some 10 units were being recorded simultaneously with a combined rate of 150 impulses s⁻¹. The output of a rate meter with a time constant of 3.3 s is recorded. At the interruption of the line, 2% lignocaine a local anaesthetic, was perfused through the nerve neuroma chamber in the left record and directly injected into the intact sciatic nerve in the right hand record. Following the injection into the neuroma chamber there is a brief rise of firing rate which is due to the pressure distortion produced by the injection and which was also observed if saline was injected. This is not observed on the normal side. After some seconds on both sides the firing rate drops to 20–30 impulses s⁻¹. The impulses which were not anaesthetised by the local block originated from proximal muscle and not from the normal or abnormal sciatic nerve. Time mark 24 s.

c—Recording as in (b) of the rate of ongoing activity in rootlets originating from a neuroma and from normal sciatic nerve. At the interruption of the line, the central cut end of the rootlet was stimulated at 100 s⁻¹ with maximal 0.1 ms square waves for 6 s. During this period antidromic impulses swept past the recording electrodes and entered the peripheral nerves. After the end of the tetanus, the firing rate of impulses from the normal endings rapidly returned to their control level. But, the impulses from the neuroma were depressed for a long period after the end of the tetanus.

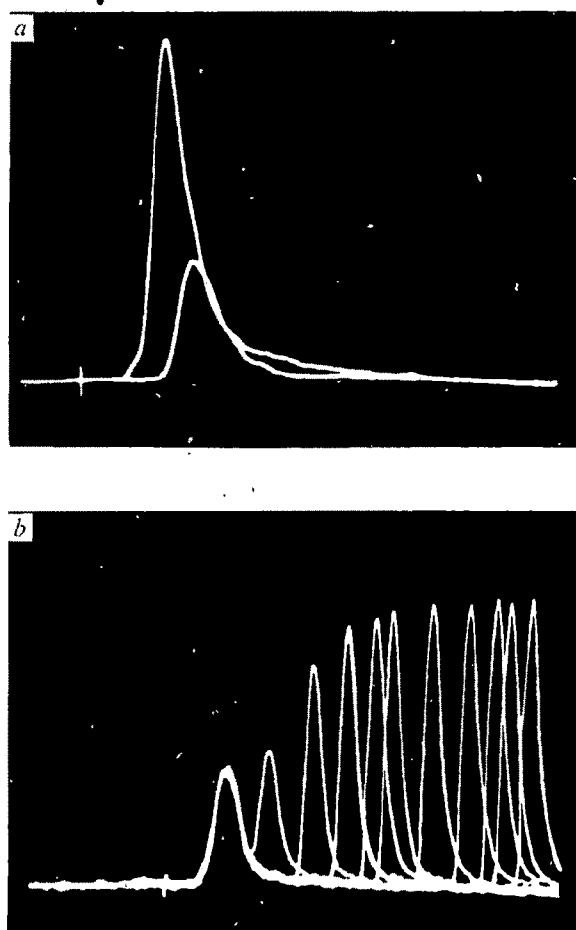


Fig. 2 *a*, Recording from a dorsal rootlet whose axons end in a sciatic nerve neuroma. Two superimposed sweeps. The slower small wave followed stimulation of the distal tip of the neuroma. The faster wave was produced by stimulating the sciatic nerve 5 mm proximal to the distal tip of the neuroma. The time difference between these two waves implies a fastest overall conduction velocity in the terminal 5 mm of 7.5 m s^{-1} . Time mark 2 ms. *b*, Interaction between the fast and slow waves shown in (*a*). Ten superimposed sweeps. For each sweep, a stimulus was given first to the extreme distal part of the neuroma and at varying time intervals following this a stimulus was given to the nerve 5 mm proximal to the neuroma tip. It will be seen that when the second wave occurs less than 3 ms after the first, it is reduced in height because the axons are partially refractory due to impulses in the same fibres which carried the earlier wave.

5 mm in a more distal direction were delayed by a further 0.66 ms giving an overall fastest conduction velocity for this terminal part of nerve and neuroma of 7.5 m s^{-1} . Since the individual fibres in the neuroma turn and twist, it is not possible to state the exact conduction distance nor the location of the stimulation. Furthermore part of the 5 mm length in the chamber is made up of relatively normal fibres. We cannot therefore give the exact conduction velocity of fibres within the neuroma but it is evident that the fibres are electrically excitable and must have a low conduction velocity, considerably below 7.5 m s^{-1} .

Evidence that the small slowly conducted wave originating from the neuroma is carried by the same fibres as the large rapid wave originating from intact proximal nerve is shown in Fig. 2*b*. Here the stimulus to the neuroma is followed at various time intervals by a stimulus to the nerve 5 mm proximal to the end of the neuroma. The recording is made from a dorsal rootlet. Ten superimposed sweeps are shown with the interval between the two stimuli changed for each sweep. The large fast wave is reduced when it approaches to within 2–3 ms of the smaller slow wave. The reason for

this reduction in height is because the impulses from the neuroma cause the axons of the central part of the nerve to be refractory. Therefore the two waves are carried in the same fibres.

Ongoing activity

If fine filaments of dorsal rootlet were teased free and mounted on recording electrodes, it was possible to record small numbers of units and to separate single units by spike height with a window discriminator. The rest of this communication deals with the responses of single axons or small groups of axons originating in neuromas. Filaments were selected which responded after electrical stimulation of the neuroma. These filaments contained fibres which were continuously active in the absence of an intentional stimulus. These impulses originated from the neuroma since they disappeared when the nerve-neuroma chamber was perfused with 2% lignocaine to produce local anaesthesia in the chamber (Fig. 1*b*). These continuously active fibres were probably myelinated afferents of relatively small diameter because the spike height of the units was less than one-third the height of the large spikes originating from muscle spindles and it is known that there is a correlation between spike height and fibre diameter⁵. If slight pressure was applied to the neuroma either by distortion of the chamber or by saline injection, the continuously active units increased their discharge rate. Previously silent fibres with higher spike heights showed a brief phasic burst of spikes during the onset of the pressure increase. Unmyelinated afferents were not examined. Filaments of ventral roots whose axons terminated in the neuroma were examined and were found not to contain ongoing antidromic impulses from the neuroma. Antidromic impulses could however be produced in these ventral root fibres by pressure on the neuroma.

It was noted that if the neuroma was driven to a high level of activity by direct repetitive electrical stimulation, the ongoing activity ceased after the stimulation. This is a phenomenon of considerable interest but since direct stimulation might have complex local effects under the stimulating electrodes, a method of activating the terminals was used in which only antidromic impulses were used. The central cut end of the filament from which recordings were made was placed on stimulating electrodes while recording electrodes were placed more distally on the same dorsal root filament.

The impulses originating from the neuroma were recorded and fed into a rate meter and a control period was recorded (see Fig. 1*c*). The filament was then stimulated maximally with 100 s^{-1} 0.1 ms square waves for 6 s. This tetanic barrage of nerve impulses swept antidromically past the recording electrodes and entered the neuroma. Following this tetanus, it will be seen that the rate of discharge from the neuroma fell and remained low for the duration of the record. The rate of spontaneous firing was depressed for minutes in all units examined and for some units, the resting discharge did not return for more than an hour. These prolonged suppressions of firing were never observed from any intact sensory endings whose excitability and ongoing discharge returned to control levels within seconds of such an antidromic invasion.

Since a tetanus is known to hyperpolarise central terminals^{6,7}, it was thought possible that this also occurs with a high-frequency antidromic invasion of a neuroma. This was tested by electrically stimulating the neuroma at 2 s intervals with a submaximal stimulus and recording the height of the orthodromic compound action potential. It was found that the electrical excitability of the neuroma decreased following a 10 s antidromic invasion at 100 s^{-1} . This decreased excitability lasted between 50 and 110 s whereas the decrease of spontaneous activity lasted in these cases between 2 and 6 min. The shorter duration of the assumed hyperpolarisation of the neuroma compared with the dura-

tion of the decreased spontaneous activity does not necessarily mean that the two are unrelated because the electrical stimulation may have triggered impulses in more proximal and larger axon sprouts than the finer more distal regions from which the impulses might originate. It might be expected since the nerve sprouts in the neuroma are fine and intertwined that they might make contact with each other so that ephaptic interactions might occur between the fibres. To test this, recordings were made from one dorsal root filament while maximal single or tetanic stimuli were applied to the entire remaining dorsal root so that the neuroma would be invaded by a large mass of nerve impulses in many fibres other than the nerve fibres from which recordings were being made. No signs were observed of any excitation or change of the spontaneous activity in fibres which had not carried the antidromic volleys and it is apparent that no obvious interaction occurs between the sensory fibres in a neuroma. Similarly, maximal stimulation of a ventral root failed to produce an afferent discharge in the dorsal root or *vice versa*. A final difference of the generation of nerve impulses from a neuroma in contrast to normal spontaneously active endings was that the discharge stopped within seconds of cardiac arrest whereas normal endings continue to discharge for many minutes.

Therapeutic implications

It is evident that these neuromas were the source of a steady barrage of nerve impulses in certain nerve fibres and that the generator of these impulses showed unusually fragile properties, being inactivated by a tetanus and being highly dependent on blood flow. The preparation with its possibilities for perfusion is an *in vitro-in vivo* isolated group of nerve endings in which it should be possible to study the action of the ionic and chemical environment on impulse generation. In addition, the results have certain therapeutic implications. If nerve impulses of the type reported here are a source of pain in man with damaged peripheral nerves and if the generator of these impulses has a lower safety factor than that of impulses from healthy nerve endings, then it is possible that drugs might be developed which affected the pathological generator without affecting normal tissue. Of more immediate interest is the use of electrical stimulation of peripheral nerve for the control of pain. This was initiated in 1967⁸ and is being widely developed. The rationale for the therapy was the suggestion that massed stimulation of peripheral fibres of large diameter set up central inhibition and closed a central gate mechanism⁹ which decreased the

transmission from the periphery to central pathways of impulses which generate pain. It was shown that certain patients were relieved of their pain not only during the tetanic submaximal stimulation of their peripheral nerves but for long periods after the end of the stimulation whereas other patients were relieved only during stimulation. The long lasting relief was a puzzle because no prolonged inhibition of spinal cord transmission was observed in animals after stimulation ceased. Now we may have an explanation. It may be that the prolonged relief which occurred in patients with damage to peripheral nerves was not produced by central inhibition but by the antidromic invasion of the damaged nerve which contained fragile generators of impulses of the type reported here. The suggestion has been made before that part of the analgesia produced by peripheral nerve stimulation was due to peripheral nerve block⁹. This suggestion was, however, based on studies of normal subjects in which, in spite of the extremely intense (20 times threshold) stimuli which were used, no clear evidence was presented that the periphery was in fact blocked. This raises the question of how such intense stimuli are tolerated and the most likely explanation is that the preceding and concomitant stimulation of large fibres sets up central inhibitions which reduces the transmission of impulses from smaller pain-producing fibres. There is considerable physiological evidence that central inhibitions are generated by repetitive peripheral nerve stimulation¹⁰. It is now apparent, however, that in certain patients who respond with prolonged relief to stimulation of a damaged nerve and who show no relief when neighbouring nerves are stimulated, there may be a turn-off of a peripheral source of pain as well as some central inhibition.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Seismic source in East Kazakhstan

ON May 1, 1969 a seismic event occurred in East Kazakhstan which, on the widely accepted body wave magnitude (m_b): surface wave magnitude (M_s) criterion^{1,2}, would be identified as an explosion; $m_b = 4.9$ and $M_s = 2.4$ (ref. 3), giving a difference of 2.5, which is typical of explosive sources. For earthquakes $m_b - M_s$ is usually less than 1.0 magnitude unit. At several teleseismic stations the P seismograms from this event recorded two clear arrivals separated by 7.5 s, which

might indicate two explosions fired in succession. At some of these stations, however, the second arrival is clearly of opposite polarity to P and is thus probably the surface reflection pP, indicating a depth of focus of about 25 km. As this depth is outside the range of routine drilling techniques the event has been tentatively identified as an earthquake³. We show here that it definitely was an earthquake.

Figure 1 shows the P seismograms (*b, e, h*) for the event, from Yellowknife, Canada (YKA), Warramunga, Australia (WRA) and Gauribidanur, India (GBA). The YKA and WRA seismograms show two arrivals 7.5 s apart; at WRA the second arrival seems to have reversed polarity relative to P, but the YKA record shows no obvious reversal. Deconvolving to remove the effects of anelastic attenuation and

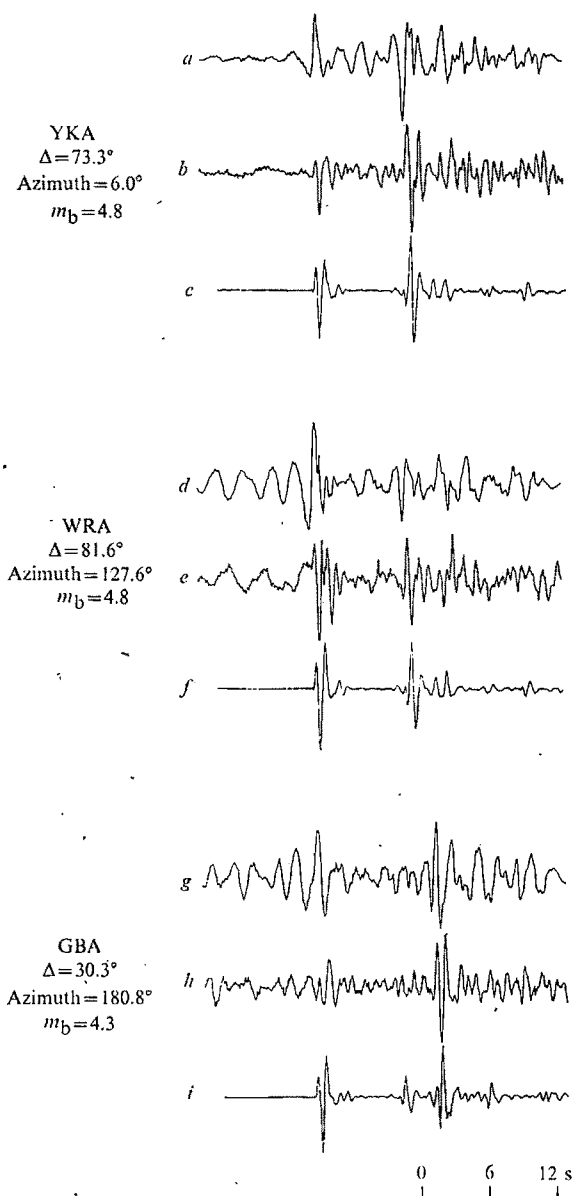


FIG. 1 Seismograms for the East Kazakhstan event of May 1, 1969 (origin time 04 h 00 min 08.7 s). YKA, Yellowknife: *a*, deconvolved trace; *b*, observed trace; *c*, theoretical trace. WRA, Warramunga: *d*, deconvolved trace; *e*, observed trace; *f*, theoretical trace. GBA, Gauribidanur: *g*, deconvolved trace; *h*, observed trace; *i*, theoretical trace. Theoretical seismograms were computed for a dip-slip double couple source, with fault plane dipping at 50° towards YKA.

of the recording system⁴ shows, however, that the second arrival is clearly reversed relative to the first at both YKA and WRA (Fig. 1*a* and *d*) and is thus probably pP. The deconvolution was made assuming a value of $T/Q = 0.2$ s, where T is the travel time and Q the attenuation factor.

The seismogram from GBA, although rather noisy, also shows two arrivals, but the separation of these is about 10.0 s, so that the second arrival at GBA cannot be pP if that arrival has been correctly identified at YKA and WRA. At GBA pP must be absent or at least very small. The deconvolved GBA record is rather difficult to interpret because of the noise but again it seems that the second arrival is reversed relative to the first.

- As the second arrivals at all three stations are of reversed polarity relative to the direct P, they are almost certainly surface reflections. If so, the depth of focus is too great for the source to be an explosion. We can however obtain further confirmation that the event is an earthquake, by modelling the seismograms. We have described⁵ the use of this technique

to model the YKA seismogram for another explosion-like earthquake which occurred about 100 km from the event of May 1. We showed that this YKA seismogram could be understood by assuming that the earthquake source was a dip-slip fault of finite size with fault plane dipping at about 50° to YKA. On this model the computed seismogram agrees closely with the observed seismogram. The YKA seismogram for the May 1 event is similar to that used in our earlier study, suggesting that the source mechanisms of the two events are similar. We have in fact used exactly the same model to compute theoretical seismograms for the May 1 event as we used earlier, except that in order to fit the P-pP delay time, the source depth has been increased from 19.4 km to 25.4 km. In addition the depth of the Moho has been increased from 23.4 km to 31.6 km, to keep the source in the crust. Figure 1*c*, *f* and *i* shows the theoretical seismograms. Agreement between theory and observation is good; in particular the model predicts the large pP at YKA and WRA and the small pP at GBA. The large arrival at GBA 10 s after P is evidently sP as predicted by the model. Because PcP is not included in the theoretical calculations, its presence in the observed seismograms should be ignored when comparing theory and observation.

Finally, the deconvolved seismograms show that the shapes of the P and pP pulses at YKA are different (Fig. 1*a*): for P the leading edge is steeper than the trailing edge, whereas for the pP the reverse is true. These differences in shape between P and pP can be explained in terms of a spreading fracture (see ref. 5). For an explosion, P and pP should have the same shape. On the simple source model assumed here, differences in pulse shape similar to those seen at YKA should be seen in reverse at GBA, which is on an opposite azimuth from the source. Furthermore the trailing edge of P should be sharper than the leading edge and *vice versa* for sP. In fact the deconvolved seismogram for GBA does not show this predicted effect. In the source model used, however, the fracture spreads symmetrically from a point of initiation in the centre of the fault plane and this is probably a simplification which needs further study.

Nonetheless, from the evidence of the relative amplitudes and the polarity of P, pP and sP at YKA, WRA and GBA, and the pulse shapes of P and pP at YKA, we conclude that the event of May 1, 1969 is an earthquake and not two explosions. This leaves the large difference $m_b - M_s$ of 2.5 unexplained; models of the type described here have $m_b - M_s$ differences of around 1.2 (ref. 5). A detailed study of the surface waves might suggest a reason for the discrepancy but such a study is virtually impossible in this case because the wave trains are mixed with the surface waves from a large ($M_s = 5.6$) Tongan earthquake. Because of this mixing it is not even certain that the M_s estimates for the earthquake of May 1 are reliable.

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Cyprus: seismic studies at sea

THE experiments described here were carried out in October 1971 on MV *Researcher* during a cruise in the eastern Mediterranean with personnel from the Department of Geodesy and Geophysics, University of Cambridge. Figure 1 shows the location of reflection surveys G and H, planned as a detailed study of the south-western boundary of the alleged Turkish plate, which is drawn by McKenzie as an arc around the south side of Turkey^{1,2}. Two short refraction lines X and Y were placed in Famagusta Bay and Morphou Bay respectively to trace the extent of the rock formations already distinguished by seismic methods on land⁴.

The reflection system comprised a 30 cubic inch air gun fired at 2,200 pounds per square inch at 10 s intervals into a Géomécanique hydrophone array with three active sections each 100 m long. An 8 kJ sparker was also used. The refraction experiments used the single ship radio sonobuoy technique with Cambridge radio telemetric buoys⁵.

The surveys carried out close to Cyprus penetrated only 0.6 s of sediment during sparker runs on G₃, G₄ and Grid H, but reached 2.5 s when the air gun was used on G₁ and G₂ (Fig. 1) (two-way times are quoted throughout). On Grid G there is an undulating upper reflector, A, which occurs within the unconsolidated sediments. It lies above a horizon, B, which is disturbed by folding, and which is 1.5 s below the sea floor. Below this there is a more intensely folded horizon, C, at 2.5 s (Fig. 2). B is probably the top of the 3.0 km s⁻¹ layer observed on refraction line X in Morphou Bay, and C can be correlated with the top of a 4.4 km s⁻¹ layer previously recognised as a limestone bed⁶, but correlated here with pillow lavas (Table 1).

Sediment thicknesses seem to be greater in the north and east towards the continental rise of the Gulf of Antalya, where deep basins occur. There is evidence of quite recent tectonism in this area, with depositional and compaction processes tending to smooth out the surface topography. There are some rather unusual topographic features along G₄: lenses or domes 2–6 km long show internal stratification, and the adjacent sediments are transparent (Fig. 3). It is possible that these domes represent olistoliths transported over a long distance from the Turkish shelf. Alternatively, they may be local sediment lenses, isolated by current action.

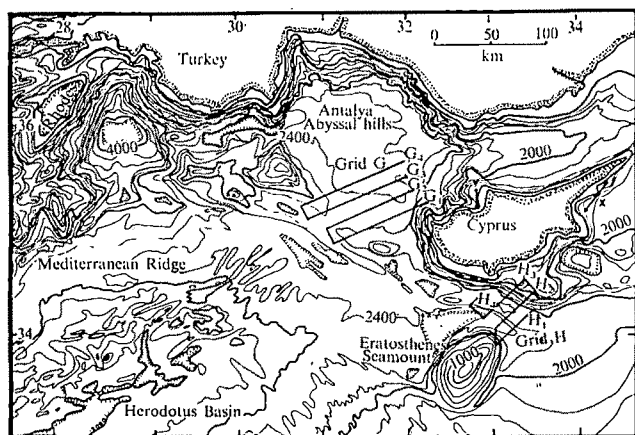


FIG. 1 Location of seismic experiments near Cyprus, superimposed on bathymetry of Carter *et al.*, 1972 (ref. 3). Contour interval 200 m.

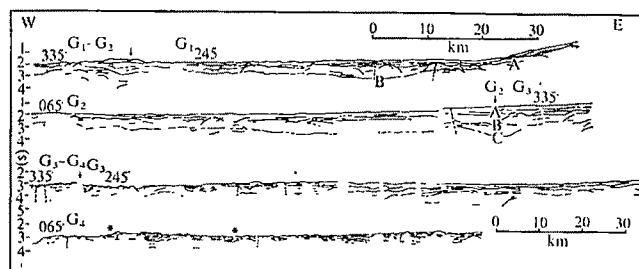


FIG. 2 Line diagrams of reflection profiles on survey area G drawn from west to east. Two-way times are in s, the arrows mark course alterations.* Enlarged section shown in Fig. 3.

Further to the east, transparent knolls 10–40 m high alternate with stratified subsiding basins. The layers thin out against the steep margins of the knolls, indicating that the latter are rising, so it is possible that their origin is diapiric. In this case, the other stratified domes may be areas of marginal sediment deposition which have been isolated by the subsidence of larger knolls or possibly saliferous diapirs 8 km across, due to solution effects, and thus producing the features shown in Fig. 3. Over Grid H a sequence of basins and swells similar to that of Grid G can be identified (Fig. 4) where the rocky basement is first submerged below ponded sediment and then rises to form a hummocky bot-

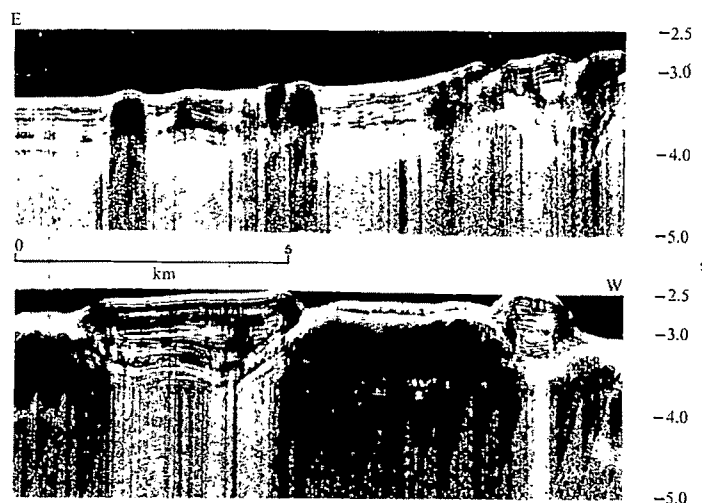


FIG. 3 Topographic features on G₄ showing diapiric type structures, and stratified domes* to the west. The position of the section is marked* in Fig. 2. Two-way times are shown.

tom topography. Two reflectors are recognised at the north-eastern end of lines H₁, H₂ and H₃, which may correspond to the upper boundaries of the layers with refraction velocities of about 3 and 4 km s⁻¹ observed on refraction lines X and Y.

A correlation of the areas of deformation occurring over survey areas G and H was attempted, and it seems that a zone of deformation exists on the western side of the lines, which coincides with the areas of deepest water (Figs 1, 5). The deformation possibly represents a north-west-south-east trending fault, described by Giermann⁷ and Wong *et al.*⁸, but at the northern end the deformed area lies further to the west than these authors have proposed. Widespread tectonic activity seems to be associated with this faulting, because the structural features already described on survey G lie immediately behind and to the east of the fault. The deformed area falls within a broad band of seismic events. Furthermore, on both grids G and H there is good correlation between a broad negative magnetic anomaly and the fault zone, the negative anomaly separating two areas of

TABLE 1 Correlation between velocities measured for Cyprus on land and at sea (sources are indicated by the references)

	Velocity on land (km s ⁻¹)				
Outcrop ⁴	In formation ¹⁰	Laboratory ¹¹ (0-2 kbar)	Velocity at sea (km s ⁻¹)	Formation	Correlation with oceanic crust ^{12,13}
2.9	3.3	2.9-4.3	2.1	Upper sediments	Layer 1
3.7			3.3	Pillow lavas	Layer 2
4.8			4.4, 4.5		
4.9, 5.1	5.1	4.9-5.7	4.4	Basal group	Layer 2
5.5	6.4		5.5	Diabase	Layer 3
			6.5	Gabbro	Layer 3

positive anomalies to the north and south. Thus the fault is indicated by reflection, seismicity and magnetic data. If the western boundary of the Turkish plate is delimited by this fault, then the boundary would pass from the west coast of Cyprus, north-west towards Rhodes and the Gulf of Fethiya (Fig. 5), rather than due north to the Gulf of Antalya as drawn by McKenzie^{1,2}.

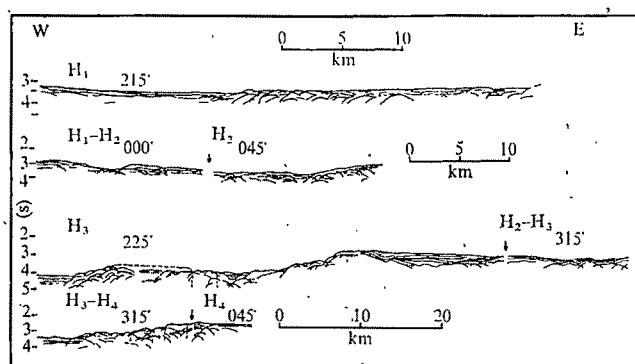


Fig. 4 Line diagrams of sparker reflection profiles, survey H, drawn from west to east. Two-way times are shown.

There have already been refraction experiments on land to measure the seismic velocities at outcrop of rocks in the Troodos complex⁴. In an attempt to correlate these with velocities at sea two short lines X and Y were placed in Famagusta Bay and Morphou Bay. These repeated the seismic experiments of HMS Challenger in 1952 (ref. 6), but over a greater range.

Results are presented in the form of travel-time graphs and structure profiles (Figs 6, 7). The structure on line X seems to dip down towards the south, because apparent

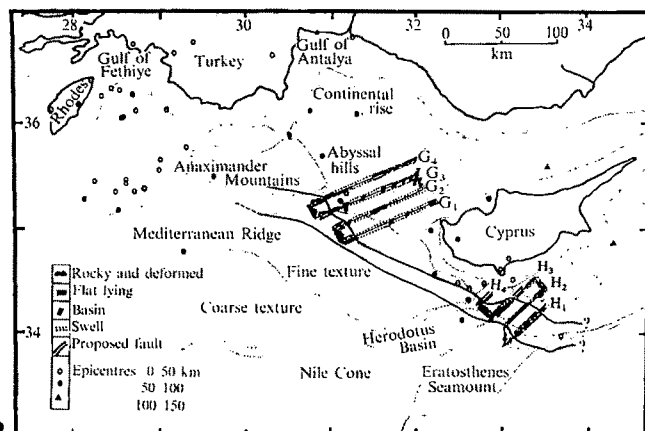


Fig. 5 Correlation of provinces over G and H to show areas of deformation, which correspond to a negative magnetic anomaly, and which possibly constitute a fault zone. Earthquake epicentres are shown. Physiographic provinces after Lort (1972) (ref. 14).

velocities along X-R, shot from north-east to south-west, are substantially lower than on X. A layer of 5.5 km s⁻¹ is detected when shooting from the north end, whereas a 6.5 km s⁻¹ layer is reached at the southern end. Otherwise, the velocities reverse to give a dipping layer solution as shown in Fig. 7a with velocities of 2.1, 3.3 and 4.4 km s⁻¹. On line Y the topography is extremely irregular so that a large scatter is observed in the arrivals and topographic corrections were necessarily large. A velocity of 2.8 km s⁻¹ is detected from second arrivals only on line Y, (A' in Fig. 6b) which is in fact a multiple of the 3.0 km s⁻¹ refractor by reflection within the upper thin sediment layer. The structure profile is shown in Fig. 7b, with velocities of 1.7, 2.9 and 4.5 km s⁻¹. The last layer was only reached on the reverse line Y-R. A comparison of the Researcher results

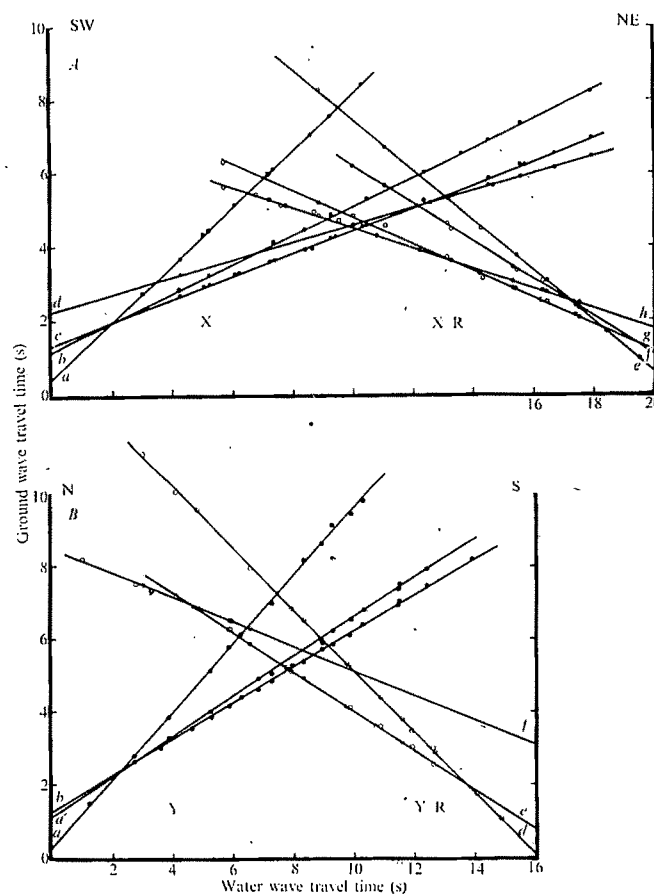


Fig. 6 Travel time graphs for refraction profiles X (6A) and Y (6B). Equations of the times are in the form: intercept time (s) + x/velocity. 6A; a, 0.4 + x/2.0; b, 1.2 + x/3.8; c, 1.3 + x/4.9; d, 2.3 + x/6.5; e, 0.6 + x/2.2; f, 1.1 + x/2.9; g, 1.2 + x/4.1; h, 1.8 + x/5.5. a, b, c, d, from profile X; e, f, g, h, from profile X-R. 6B; a, 0.3 + x/1.6; a', 1.1 + x/2.8; b, 1.2 + x/3.0; d, 0.8 + x/1.8; e, 0.8 + x/2.8; f, 3.1 + x/4.5. a, a', b, from profile Y; d, e, f, from profile Y-R.

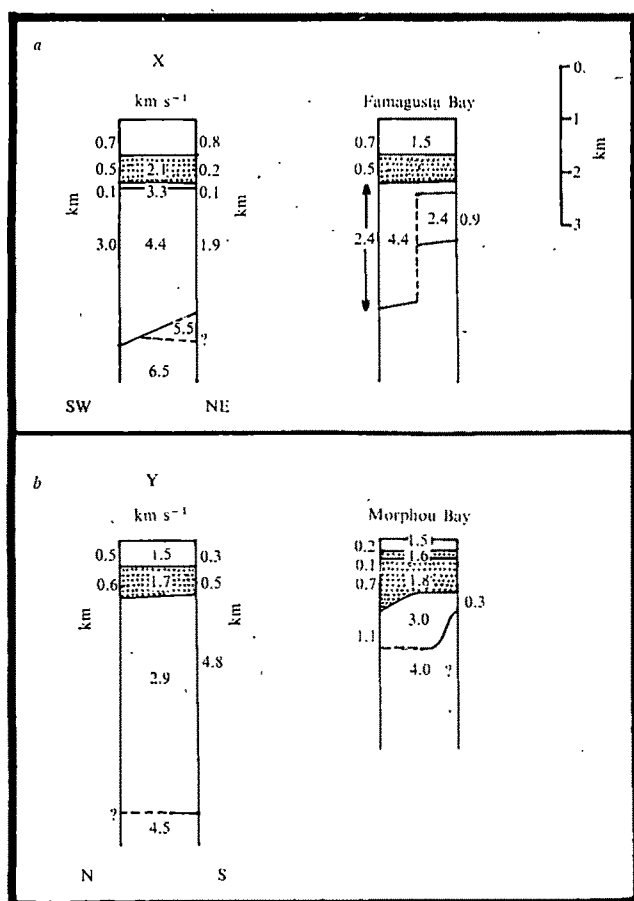


FIG. 7 Comparison of crustal structure profiles near Cyprus obtained by HMS Challenger in 1952 (ref. 6), and MV Researcher in 1971. *a*, line X in Famagusta Bay; *b*, line Y in Morphou Bay. The layer thicknesses are calculated at each end of the profiles as indicated.

with those of Challenger shows a similarity in general structure but deeper penetration on profiles X and Y-R (Fig. 7). A correlation between velocities measured on land and at sea can be seen in Table 1. On line Y, the 4.5 km s⁻¹ layer is tentatively correlated with the pillow lavas which also outcrop on the southern edge of the bay⁹. They are supposedly quite thick as no material of greater velocity is detected even at a range of 22 km. The velocities of 5.5 km s⁻¹ and 6.5 km s⁻¹ probably correspond to layer 3 velocities.

It is apparent from the table that velocities measured in the rocks¹⁰ at outcrop⁴ are generally lower than those obtained in the laboratory¹¹ or at sea. This is undoubtedly an effect of the difference in confining pressure on the rock units in the two environments^{4,10}, and also of the shattered nature of the outcropping units. These results are thus compatible with the widely accepted proposal that Cyprus is a section of oceanic crust¹⁰⁻¹³.

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Flysch-ophiolite successions: polarity indicators in arc and collision-type orogens

Arc systems formed above subduction zones show a characteristic Benioff zone along or within which the subducting plate descends. In modern active arcs the polarity is indicated by the relative position of the volcanic arc and trench, by changes in the K₂O/SiO₂ ratio across the volcanic arc¹, and commonly by arc curvature. The polarity of ancient arcs can be determined by variations in the K₂O/SiO₂ ratio in volcanic arcs or their plutonic granodioritic equivalents², by recognition of parallel ophiolite and magmatic belts, or by the presence of paired metamorphic belts³. The polarity in many ancient arc systems, and also in collision-type orogens, is, however, uncertain either because magmatic arcs are poorly developed or because the relative positions of the paired belts have been confused by tectonic movements.

I suggest here that in some arc systems, the polarity can be determined by the predominant younging direction of flysch-ophiolite successions. In collision-type orogens, the polarity is indicated either by the younging direction of the flysch or by the direction from which the flysch is overthrust.

Recent work on Barbados⁴ provides evidence of the mechanism of underthrusting and deformation within pelagic sediments and turbidites deposited on the ocean floor and carried into the subduction zone. As these flysch-type sediments approach the subduction zone a thrust plane inclined towards the zone develops, cutting the sediments at a low angle (Fig. 1a). Continued subduction and underthrusting results in emplacement of the sediments overlying the thrust plane in a tectonic wedge above the subduction zone. Younger sediments deposited on the subducting plate further away from the plate boundary are in turn cut by a further thrust plane as they near the subduction zone, and are underthrust by still younger sediments. A tectonic pile of sedimentary wedges results, in which the overall age of the flysch increases towards the over-riding plate. Within each tectonic wedge, however, the age of the sediments decreases in the same direction (Fig. 1b).

Major belts consisting largely of late Mesozoic or Cainozoic flysch, deformed in a style similar to that of Barbados, occur in some other active or recently active arc systems, for example the Sunda-Burma arc⁵, southern Alaska⁶ and north-west Borneo^{7,8}. Within these belts part of the ophiolite sequence serpentine-gabbro-dolerite-pillow lavas, overlain by radiolarian cherts, is locally present beneath the flysch, forming a 'eugeosynclinal' association of lithologies. The ophiolite sequences can be interpreted as part of the oceanic crust from the subducting plate, scraped up and emplaced together with the overlying flysch above successive thrust planes (Fig. 1c).

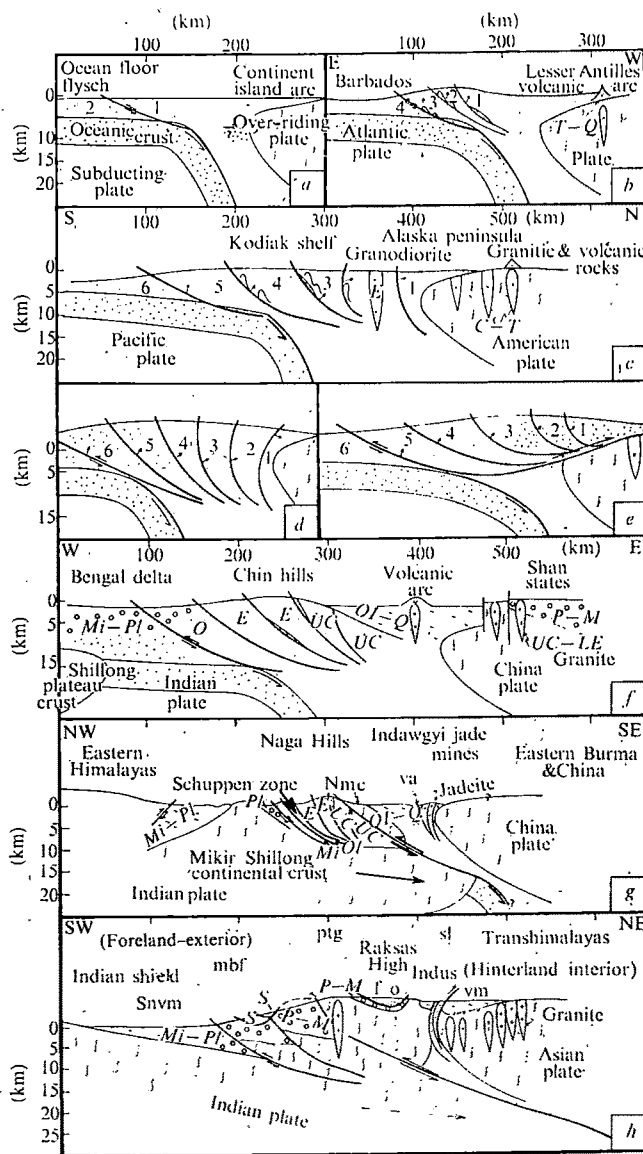


Fig. 1 Schematic simplified cross sections through Cinozoic arc systems and collision-type orogens. Locations of cross sections shown in Fig. 2. *a*, Initial underthrusting during start of subduction (no known example); *b*, Barbados (modified from Westbrook *et al.*⁴); *c*, southern Alaska showing plutons intruding flysch (modified from Moore⁹ and (Martin)¹⁹; *d*, overturning of flysch due to progressive steepening of thrust units (no Cinozoic example known); *e*, tectonic emplacement of flysch on overriding continental margin (no Cinozoic example known); *f*, Chin Hills, Upper Burma (Modified from Brunnschweiler¹⁴ and others (references in Mitchell and McKerrow)²⁰; *g*, Naga Hills, Upper Burma (Modified from Brunnschweiler¹⁴; Nmc, Naga metamorphic complex; va, volcanic arc; *h*, Kumaon Himalayas. (Modified from Gansser)¹⁶. Alpine terminology in parentheses; Snvm, Siwaliks non-volcanic molasse; mbf, main boundary fault; ptg, post-tectonic gneiss; f-o, flysch and ophiolites; sl, suture line; vm, volcanic molasse. Possible sequences of events in one arc system: *a* → *b* → (*c* or *d*) → *f* → *g*; *a* → *e*; *a* → *b* → *f* → *h* if flysch absent throughout subduction. ~, fluviatile and molasse facies; open circles, shelf or delta top facies; heavy dots, ophiolites; faint dots, flysch; S, continental crust of subducting plate; f, continental or island arc crust of over-riding plate. 1, 2, 3 . . . , successively younger flysch units. Arrows indicate younging direction. P, Palaeozoic; M, Mesozoic; C, Cretaceous; T, Tertiary; E, Eocene; Ol, Oligocene; Mi, Miocene; Pl, Pliocene; Q, Quaternary; L, lower; U, upper.

Within some flysch-ophiolite belts granodioritic or granitic intrusions slightly younger than the flysch are present. An example occurs in southern Alaska, where early Tertiary

granodiorites intrude the older part of a belt of tectonically repeated late Mesozoic and Cinozoic flysch with minor ophiolites. Here the flysch youngs mostly northwards within each tectonic unit. These 'post-tectonic' plutons represent magmatic arc activity above a Benioff zone which either increased its inclination or migrated oceanwards relative to its position during the Cretaceous⁹ (Fig. 1c).

An example of a deformed flysch-ophiolite succession in an ancient orogen is provided by the Lower Palaeozoic succession of the Southern Uplands in Scotland, where imbricate successions of north-westward-dipping turbidites overlie pillow lavas and cherts¹⁰. In Ireland, in part of the western continuation of the Southern Uplands the turbidites are overturned to the north (A. E. Phillips, personal communication). This overturning possibly resulted from progressive steepening of the already emplaced tectonic wedges during underthrusting by younger sediments (Fig. 1d).

In the southern Alaska flysch belt there is evidence that the thrust planes converge downwards towards a zone inclined beneath the continent⁶. It is possible, however, that in some other arcs the thrusts could converge in a plane of decollement, convex downwards, resulting in overthrusting of the flysch belt onto the continent (Fig. 1e). A possible example of this is found in the Patagonian Andes, where Mesozoic flysch is thrust eastwards over a miogeosynclinal succession and volcanic belt¹¹.

The flysch successions considered can be interpreted as submarine delta fan deposits which accumulated mostly on the ocean floor and perhaps partly in a trench. In some arc systems in which little flysch reaches the ocean floor, for example the Ryukyu Arc, a thick succession with a seismic velocity typical of sediments is present on the arc side of the trench¹². This succession possibly consists largely of pelagic sediments tectonically emplaced in a similar way to the flysch, although analogous thick belts of tectonically repeated pelagic successions are rare in ancient orogens.

In the Cinozoic arc systems considered, the polarity is invariably indicated by the predominant younging direction of the flysch within each thrust slice, which is towards the over-riding plate. The flysch is commonly tightly folded and overturned adjacent to, and particularly above, thrust planes. But where the thrust planes themselves are overturned as a result of oversteepening the flysch still youngs towards the over-riding plate.

Evidence for successive underthrusting of flysch-ophiolite wedges in arc systems suggests that a similar process could affect continental crust and overlying shelf sediments which are underthrust prior to and during continental collision.

In the Cinozoic Burman arc, which developed above an eastward-dipping Benioff zone, delta top sediments have been underthrust beneath the flysch-ophiolite belt of the Chin Hills^{13,14} (Fig. 1f). Further north, continental crust of the Indian Shield, exposed in the Mikir Hills, is underthrusting

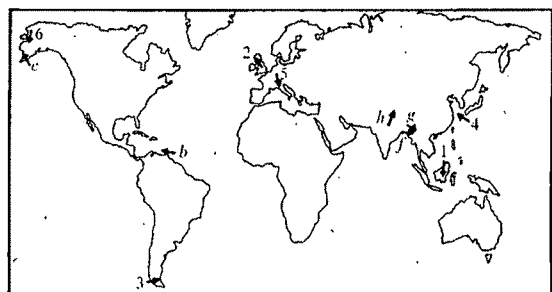


Fig. 2 Locations of cross-sections shown in Fig. 1 and of localities referred to in text. Arrow points towards known or inferred subduction direction. *b*, Barbados; *c*, southern Alaska; *f*, Chin Hills; *g*, Naga Hills; *h*, Kumaon Himalayas; 1, north-west Borneo; 2, Southern Uplands; 3, Patagonian Andes; 4, Ryukyu Arc; 5, Swiss Alps; 6, northern Alaska.

deformed flysch and ophiolites of the Naga Hills (Fig. 1g)¹⁵. Shelf sediments of the Indian plate are involved in thrusting in the west, and in the east the Naga metamorphic complex is thrust westwards over the flysch¹⁴. The metamorphic complex can most simply be explained as an upthrust slice of the continental Indian plate. This requires that the continental crust of the Indian plate has overridden the original subduction zone, the position of which is shown in Fig. 1f, and underthrust the continental part of the China plate (Fig. 1g). The belt of jadeite-albite dykes and glaucophane schists at Indawgyi Lake can then be interpreted as oceanic crustal rocks dragged downwards along or above the Benioff zone, and subsequently thrust upwards together with the metamorphic complex.

Although flysch is largely absent in the Kumaon Himalayas¹⁶, the Himalayan structural setting broadly resembles that of the Naga Hills, and can be explained by northward subduction followed by underthrusting of the Indian Shield towards and beneath Tibet¹⁷. Metamorphic rocks and shelf sediments within thrust slices in the Kumaon Himalayas¹⁶ represent tectonic wedges of the Indian Shield and overlying shelf deposits, and the Raksas High, south of the Indus Suture, is possibly a tectonic slice of the Indian Shield analogous to the Naga metamorphic complex in Burma (Fig. 1h). 'Post-tectonic' granites in the Himalayas may have resulted from the final stages of subduction along the original Benioff zone, the inclination of which approached the vertical as a result of the slowing down and stopping of subduction.

In the Naga Hills collision-type orogen, as in arc systems, the polarity is indicated by the younging direction of the flysch. Where, however, the flysch either dips at low angles, or shows no predominant inclination, as in the Kumaon Himalayas, polarity is indicated by the direction of overthrusting, which is directed towards the subducting plate¹⁷.

In the complex collision belts of the European Alps, overthrust slices of metamorphic rocks and shelf sediments, analogous to those present in the Himalayas and Naga Hills, can be interpreted as tectonic wedges of the underthrusting continent on the subducting plate, rather than crustal flakes from the over-riding plate¹⁸. In the eastern Alps the northward direction of thrusting of the ocean floor rocks suggests that the polarity, or direction of underthrusting, was to the south rather than to the north as required by the flaking model¹⁸, although it is possible that, at depth, the Benioff zone dipped to the north. The Alps are complicated by major gravity slides, but the relative positions of structural units resemble those of similar units in the Himalaya¹⁹ or Naga Hills collision belts (Fig. 1g and h). The contrast between the predominant metamorphic thrust units in the Himalayas, and the flysch-ophiolite successions in the Naga Hills and many of the Alpine chains, indicates that delta fan deposits were absent in the Tethys ocean north of India during the Cretaceous and early Tertiary.

Structures similar to those formed in a continent-continent collision could result from the collision of a continent on a subducting plate with an island arc on the over-riding plate. In northern Alaska northward overthrusting of the Brooks Range miogeosynclinal succession by similar shelf facies, and by Jurassic flysch and ophiolites of the Koyukuk geosyncline to the south^{19,20}, possibly resulted from attempted southward subduction of the miogeosyncline beneath the flysch. The Tintina Trough could then represent the suture line, analogous to the Indus Suture, between the Koyukuk flysch to the north, and a late Jurassic subduction-related island arc complex in the Ruby geanticline on the over-riding plate to the south.

Flysch-ophiolite successions are present in many Phanerozoic and Late Precambrian orogens, some of which can be interpreted as arc systems and others as collision belts. The younging direction of the flysch in both types of orogen,

and the direction from which the flysch was thrust in collision-type orogens, can indicate the subduction direction and hence the polarity of parts of these orogens. Evidence for polarity, essential to interpretations of the orogens, is of increasing use in the search for mineral deposits related to magmatic arcs and of indirect use in petroleum prospecting within and adjacent to the younger orogens.

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Hot spot in France?

THE hypothesis of hot spots is central to many discussions about volcanism; it was first put forward by Wilson¹ and has just been reemphasised by Morgan² who proposes that a hot spot corresponds to the top of a plume raising from the deep mantle³. As a plate drifts above it, volcanic activity takes place, giving rise to a linear chain structure with a uniform progression of ages from the active head in a direction defined by the reversed absolute drift velocity of the plate.

The best studied example is the Hawaiian volcanic island chain. More recently, Wilson and Burke⁴ have proposed a hot spot origin for many volcanic structures on ocean floors and on continents. So the concept of hot spots cannot always correspond to a deep mantle plume but may be related to a thermal inhomogeneity located only in the asthenosphere. A uniform progression of ages along one azimuth is still expected, however, unless the plate involved is stationary relative to the mantle.

Could volcanism in France be associated with a hot spot? We do not give a clear-cut answer to this question here; rather we emphasise that no simple cinematic linear model is consistent with the ages of the volcanic provinces (Fig. 1) associated with the Massif Central. Schematically, the pattern has three branches diverging from a centre of volcanism, 20 Myr old, the Cantal; the extreme end of each branch is characterised by recent activity. Similar patterns have been

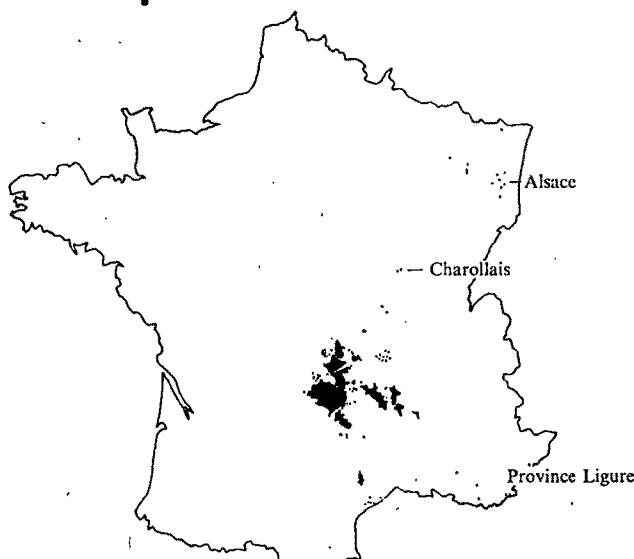


FIG. 1 Volcanism in France.

analysed by Dewey⁵. On a wider scale, however, some linear age progression is evident.

Between 20 and 13 Myr ago volcanic activity was not very prevalent. The formation of the Cantal caldera was probably connected with abundant pyroclastic emissions between 13 and 7 Myr ago. Important basaltic or trachyandesitic lava flows also took place and phonolites appeared around 8 Myr ago (ref. 14). We follow this, first, along the northern branch: the Mont-Dore started with a pyroclastic phase 8 Myr ago and true rhyolitic outflows only began 2.2 Myr ago; the activity stopped 0.4 Myr ago (ref. 15). The corresponding rectangle in Fig. 3 illustrates the geographical spread to the north and the age interval involved. Further north the Chaîne des Puys started 0.15 Myr ago (R.B., H.B., and A. Rudel, personal communication) and was still active in 80 B.C. (ref. 16). As radiogenic dating is not available for the Cézallier, the corresponding rectangle on Fig. 3 is not well determined. From its morphology, this province seems to be older than Mont-Dore.

Simultaneously, the southern branch started 13 Myr ago in Cantal, where the overall activity stopped about 3 Myr ago (ref. 12). Next came the Aubrac and then several well aligned provinces¹⁷⁻¹⁹ down to the coast near Agde, where the most recent outcrops are dated 0.7 Myr. (ref. 9). Younger outflows further south may have been buried on the crumbled floor of the Mediterranean Sea.

The third branch spreads to the south-east and is made up of two distinct echelons: the Velay-Coiron alignment (which stopped being active at 4 Myr (ref. 20)) and further south of the Devès-Ardèche, in which province the most recent basaltic flows took place about 12,000 yr ago, (ref. 21; Fig. 3).

Before describing the age and space structure of the Massif Central in more details, we note the existence of two other distinct volcanic phenomena in France (Fig. 1). In the Ligurian province, the andesitic rocks dated 26 to 29 Myr (ref. 6) originated at the end of the Oligocene and are probably associated with a past subduction zone⁷. Rift volcanism is well known on the sides of the Rhine graben (between 80 and 0.4 Myr (refs 8 and 9)). There are other graben formations to the south-east of the Rhine valley forming an *en échelons* pattern (Fig. 1): the Charollais graben also shows Eocene volcanic outcrops dating from 60 to 30 Myr ago (ref. 10); no volcanism is known in the Dombes graben. The Roanne-Montbrison graben is also volcanic, and finally the Limagne has volcanic outcrops both on its central axis (22 to 3 Myr (ref. 11)) and along its granito-metamorphic sides.

Thus subduction, on the one hand, and graben formation in connection with the alpine tectonics involving a possible shearing or extension between the Hercynian platform and the Alps, on the other, are able to explain the volcanic activity described, the total volume of which is rather modest by comparison with the Massif Central.

The spreading of volcanic activity along the three branches of the Massif Central can be analysed on the basis of Fig. 2. An initial outflow took place 21 Myr ago¹² at the limit between the north of Cantal and the Cézallier massif. This location lies at the north-west end of an Hercynian horst containing the only known peridotite residuals of the region¹³, witness of a very old (Cambrian?) suture. We take it as the geometric origin of volcanism, noting, however, that other Lower Miocene, mainly basaltic, outcrops have been dated all around the Cantal massif in direct contact with the crystalline basement.

We do not discuss here the important question of the chemical evolution of magmatism with time or structural location. The facts we stress are the following: (1) As far as volume of emission is concerned, the provinces associated with the Massif Central are the dominant feature of volcanism in France. (2) Activity has spread along three arms around

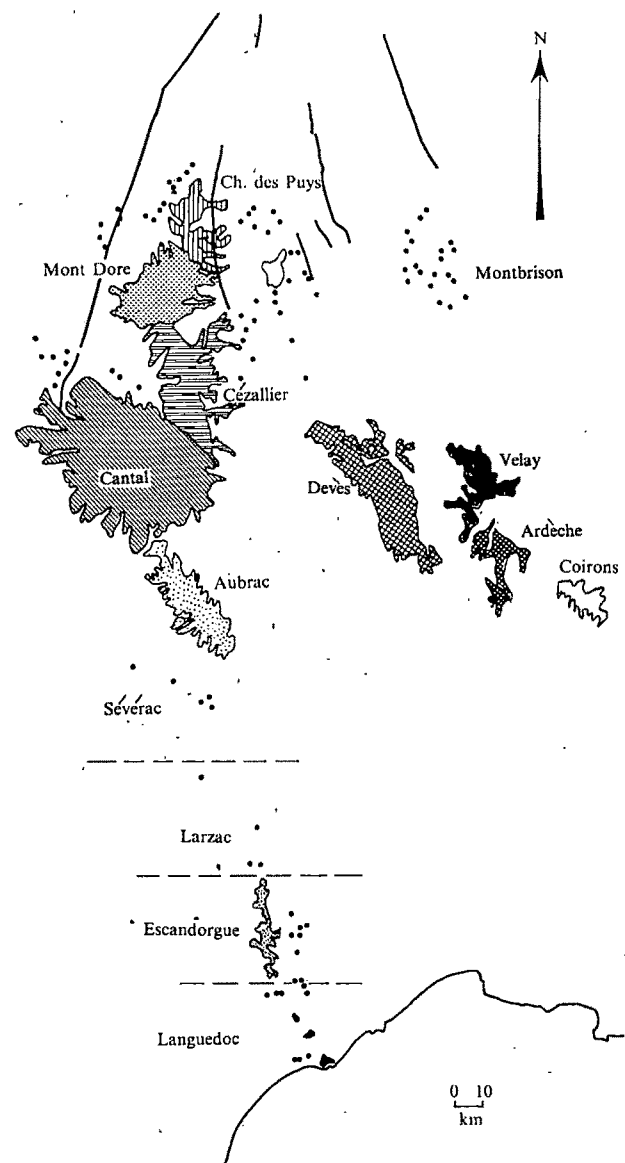


FIG. 2 Volcanism of Massif Central, Causses, Escandorgue and Languedoc. Each volcanic district is represented with a specific graphical design. Each of them is represented by the same specific graphical design in Fig. 3.

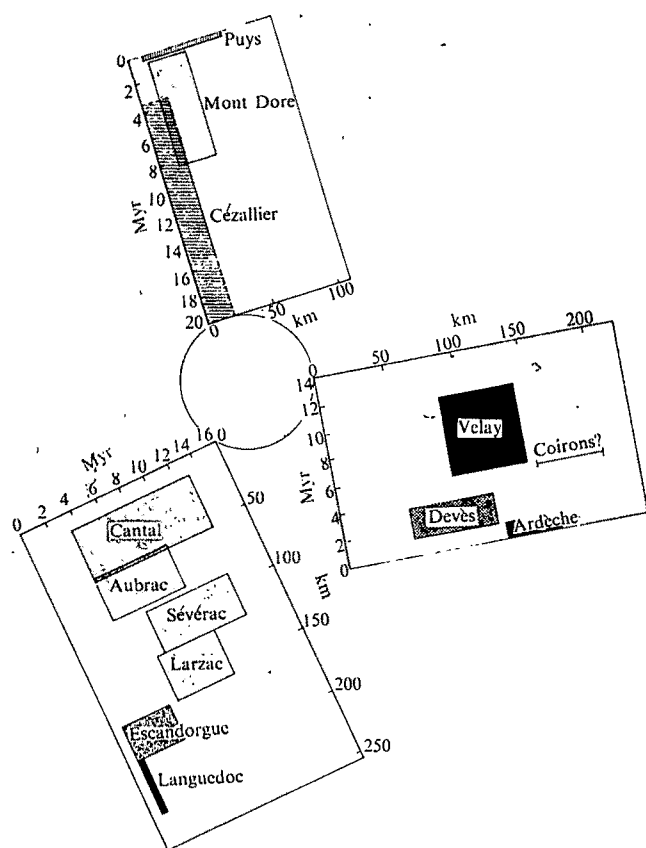


FIG. 3 The great circle represents the situation of the starting point of French volcanism 20 Myr ago. Each of the three rectangles is respectively a diagram of the three branches along which volcanism spreads. Inside the big rectangles, small ones are measured in time (Myr) representing activity within a province (duration of volcanic activity) and in km (distance of the province (proximal and distal edges) from the starting point).

the region of initial volcanism in Cantal dated 20 Myr. If this spreading is assumed to have started 13 Myr ago, the average spreading velocity can be estimated from Fig. 3 to be 0.6, 1.7 and 1.0 cm yr^{-1} along the northern, southern and south-eastern branches respectively. Although the accuracy of these figures is poor, the difference between them is real. (3) The volume of volcanic products emitted decreases approximately exponentially from Cantal to the extreme end of the three branches. For example the Cantal massif is about 10 times as big as the Mont-Dore, which is in turn about 10 times bigger than the Chaîne des Puys.

So we conclude that the age and space structure of the Massif Central does not exclude or prove the presence of hot spot under the European lithosphere in this area. It certainly does not yield a quantitative knowledge of the drift velocity of the European plate with respect to the asthenosphere beneath it.

Returning to the general distribution of volcanic centres in France, the age of initiation of rift volcanism progresses quite uniformly as one moves from the Rhine graben (80 Myr) to the Charollais (60 Myr), the Limagne (22 Myr) and finally the southern branch of the Massif Central, which lies along this general trend. It is tempting to connect this with a hot spot. The spreading velocity of the latter would be non-uniform, averaging about 1 cm yr^{-1} . Such a proposal immediately raises questions: What is the relationship between volcanic activity and tectonism? Why does the magmatic activity last for so long after the initial phase? What causes the occurrence of a paroxysm in the activity around 13 Myr connected with the initiation of a three-branches pattern? Finally, there are difficulties in reconciling this overall

north-south trend with the suggestions of Duncan *et al.*²² of an east-west progression of volcanic activity from Poland to the Eiffel and a south-north migration from Scotland to Ireland. Dating and magmatic studies on a large scale at the periphery of the Alps is vital if a comprehensive model of volcanism on the European plate is to be built up.

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Glacial and postglacial sedimentation in the Sea of the Hebrides

SHALLOW seismic profiling, sediment coring and drilling from an anchored ship have provided new information about sedimentation in the Sea of the Hebrides. Some initial results are summarized here.

On shallow seismic profiles over the inner continental shelf (east of 08°00'W, Fig. 1) a principal reflector, coincident with a stratigraphic unconformity, is interpreted as a rockhead reflection. This surface is obviously glaciated¹, overdeepened trenches are common, and where it crops out it is smoothed and striated². The layer above this reflector is, therefore, interpreted as Quaternary sediment. Its thickness has been calculated assuming a seismic velocity of 1.8 km s^{-1} and isopachytes are shown in Fig. 1. Cores and boreholes through this layer have recovered the following lithologies.

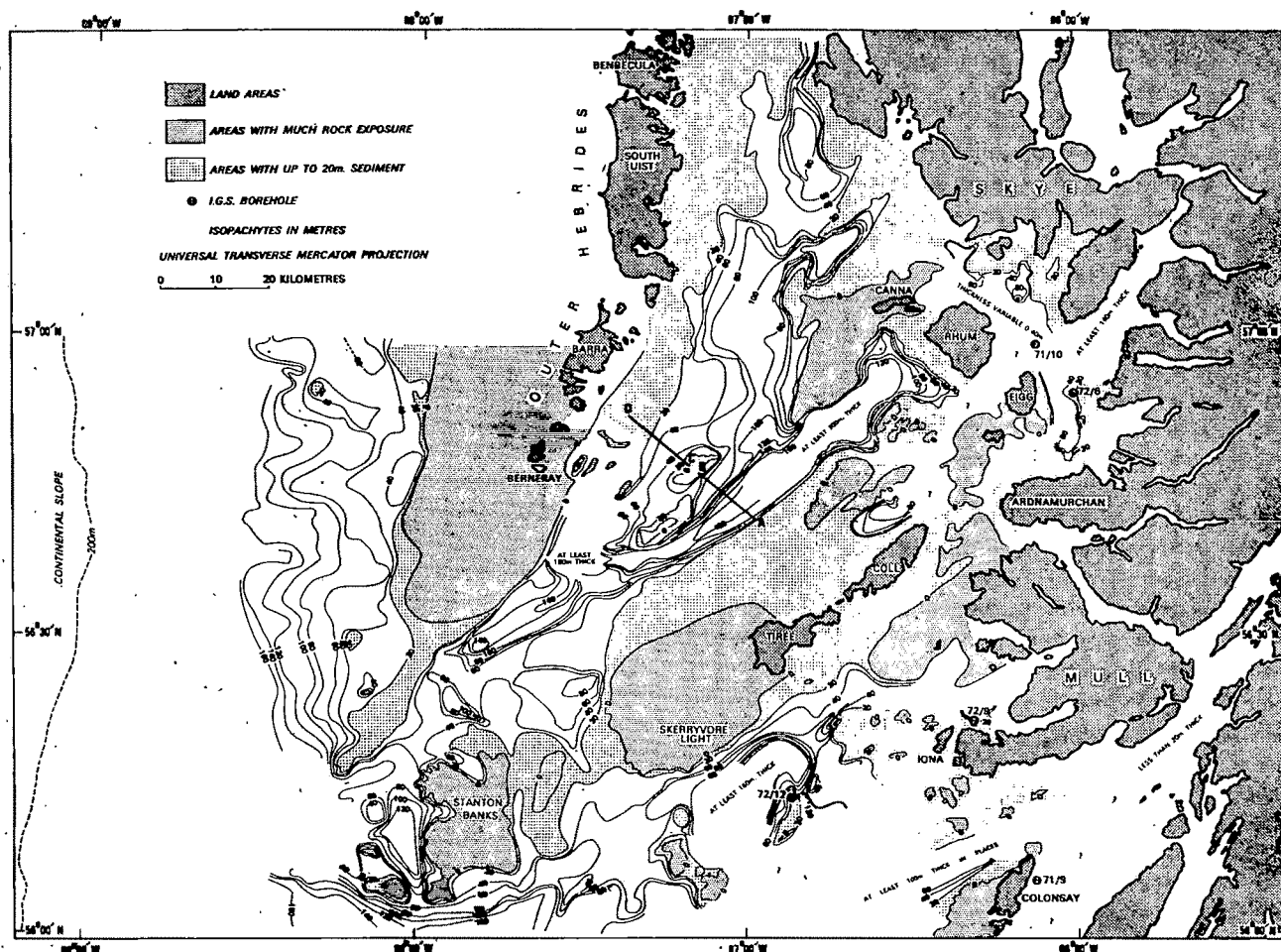


Fig. 1 Isopachytes on Quaternary sediment.

Formation 1. Till (Fig. 2) constitutes only a small proportion of the total sediment encountered in the boreholes. Reflections from the surface of moraine hummocks are seen on some seismic profiles (Fig. 3) but more commonly the profiles fail to record a boundary between Formations 1 and 2. This may be due to lack of velocity contrast between the two or to an insignificant thickness of Formation 1.

Formation 2. These firm, poorly sorted sandy muds³ (Fig. 2) contain variable amounts of granule and pebble grade lithic fragments⁴. Benthonic foraminifera are sparse but there is a significant dinoflagellate cyst population.

Lithology (compare with refs 5 and 6), stratigraphical position beneath late glacial sediments of Formation 3 and distribution on the courses of the major ice streams together suggest that Formation 2 was deposited in close association with ice. In spite of the firmness of the sediment and the presence of gravel grade material, the consistently distinctive marine dinoflagellate cyst assemblages indicate that this formation comprises marine sediment and not till. The nature of the 'till' in borehole 72/12 is not understood; it may be a submarine 'flow till'⁵ rather than a true till.

Formation 3. A pilot study of this formation in borehole 71/9 shows that the sediments are poorly sorted sandy muds³ similar to Formation 2. They are, however, softer and contain medium sand to pebble grade fragments only rarely, and then at levels coinciding with cold water faunal assemblages.

Study of foraminifera and dinoflagellate cysts has shown that the Formation 3 sequence can be divided into three palaeoclimatic zones¹. From -30 m to -28 m the foraminiferal population is dominated by *Ammonia batavus* (Hofker), a warm, shallow water species. A rich assemblage of dinoflagellate cysts indicates a similar environment. Between -26 m and -16 m a cold water assemblage of foraminifera,

dominated by *Elphidium clavatum* Cushman, coincides with poor recovery of dinoflagellate cysts. From -14 m upwards cold water foraminifera decline in importance. The dinoflagellate cysts from -10 m to the surface are modern in aspect.

The disseminated organic matter contains significant amounts of reworked Mesozoic material. The least contaminated sample was at -6 m; of the organic-walled microplankton only 14.7% were Mesozoic forms and amorphous organic matter appeared fresh. A date of $9,961 \pm 250$ BP was obtained from the disseminated organic matter at this depth (Scottish Reactor Research Centre 117). This has been taken as a maximum age which reduces to 8,680 BP on the assumption that 14.7% of the carbon is old. Assuming continuous deposition it suggests that the older 'warm' water fauna belongs to the Allerød interstadial, the 'cold' water fauna to the period of the Loch Lomond readvance stage and the younger 'warm' water fauna to the final climatic amelioration.

Formation 3 sediments are considered to have been deposited at some distance from the ice front (compare with the 'periglacial marine' sediments of southern Alaska⁷). During deposition of the sequence in borehole 71/9, ice, when present, lay behind the present coastline; a plentiful supply of sediment could be expected both from marine erosion of the sea floor and coastline and from fluvio-glacial sources. This would account for the high sedimentation rate implied by the date. A similar succession has been described on the east coast of Scotland (J. D. Peacock, to be published).

The poor recovery of dinoflagellate cysts between -26 m and -16 m may be attributed to a continuously high level of suspended matter (produced by the Loch Lomond readvance stage) and not to low water temperature. The sus-

pended matter would adversely affect the thecate dinoflagellate population and add a dilution factor resulting from high sedimentation rates⁸. In contrast the significant populations of Formation 2 are inferred to have lived in clear, cold water adjacent to floating ice. Potential suspended matter remained frozen into the ice to be released intermittently, perhaps by seasonal melting, to form Formation 2 deposits.

Formation 4. These sediments form a thin layer (0.05 m to 2.0 m) on all older formations. Their character can be related to bathymetry and situation with respect to the prevailing south-westerly swell. They are interpreted as modern sediments.

Calibration of shallow seismic profiles by the boreholes suggests that Formations 2 and 3 each produce a characteristic seismic texture which can be used to determine their distribution. Formation 3 typically has closely spaced, horizontal reflectors (Fig. 3) which fade laterally and which cannot be correlated with any visible structure in the boreholes. A second characteristic texture, interpreted as Formation 2, has widely spaced reflectors (Fig. 3). Only borehole 72/12 has passed through this unit but if the interpretation is correct then most of the thick sediments west of Colonsay are of Formation 2, as are those in the trench northwest of Coll (Figs. 1 and 3).

West of Colonsay Formation 3 sediments fill shallow depressions on the surface of Formation 2. In contrast, east of Colonsay, in an area protected from the prevailing south-westerly swell, nearly 30 m of Formation 3 sediments have been deposited and preserved at a topographically higher level (compare boreholes 71/9 and 72/12, Fig. 2). Formation 3 sediments also occur in the trench north-west of Coll (Fig. 3) and in the glacial trenches around the Inner Hebridean islands. Considerable variations in their thickness, however, suggest deposition (or subsequent erosion) under the control of a complex system of currents.

On the outer continental shelf the sea floor is relatively flat but the rockhead reflection drops evenly westwards¹. There are no trenches overdeepened by glacial action on rockhead and so the possibility that Tertiary sediments lie above it cannot be excluded.

The two seismic textures on profiles west of Colonsay are also present here. Short (0.8 m) cores taken at the outcrop of the lower unit penetrated a thin (0.1 to 0.5 m) cover of Formation 1 sands and gravels to recover Formation 2 sediments.

Evidence from the land shows that a major ice sheet originating on the mainland moved westwards to extend across the outer shelf. In contrast to this evidence, overdeepened trenches off shore trend along north-east to south-west structures¹. It has been inferred¹ that initially the area was in-

vaded by south-westerly moving ice streams from local high ground, but that during maximum glaciation the westerly moving ice sheet crossed the whole area leaving striae and erratics on areas which are now land. This sequence was reversed during deglaciation.

Deposition of the sediments would have been controlled by the nature of the ice and the manner of its retreat, in particular its relation to changes in sea level. A complex history of sedimentation is thus indicated and the reconnaissance nature of the evidence presented here allows only outline conclusions to be drawn.

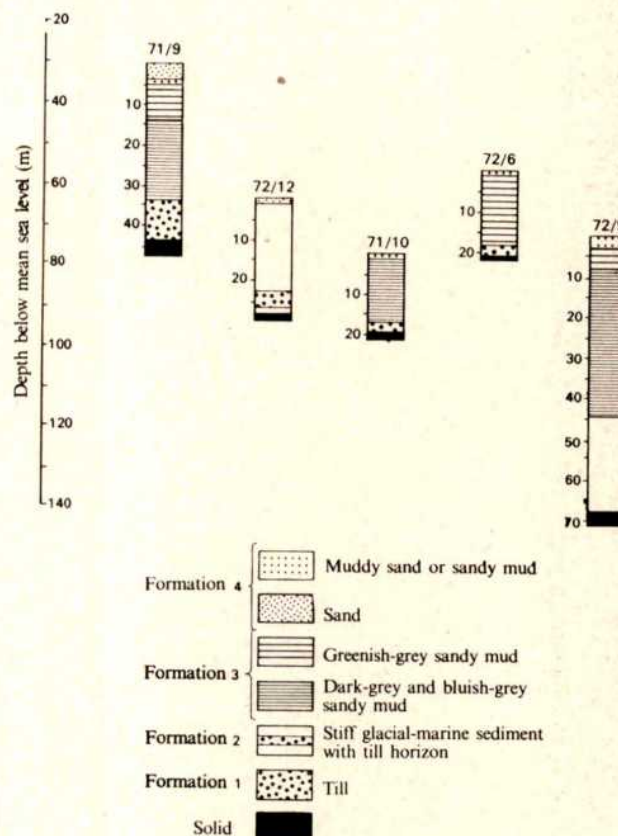


Fig. 2 Boreholes through Quaternary sediment.

It is suggested that the four formations were deposited diachronously by the retreating ice of the last (Devensian) ice sheet. The Formation 2 sediments on the outer continental shelf (Fig. 1) were deposited by the main ice sheet, and those west of Colonsay and north-west of Coll (Figs 1 and 3) by major, south-westerly moving ice streams, probably at a more advanced stage in the retreat. Their thickness reflects

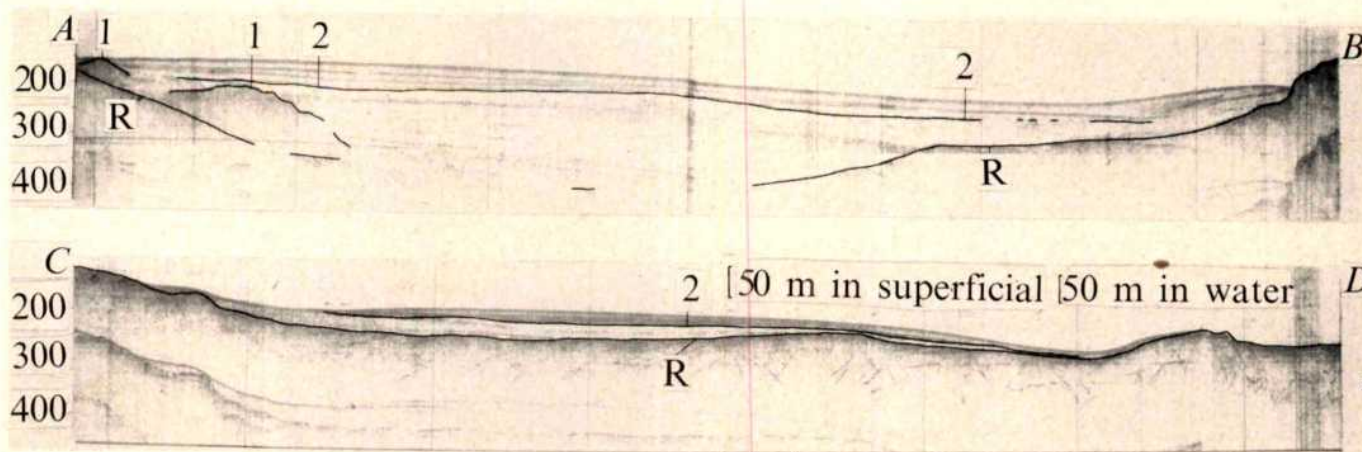


Fig. 3 Shallow seismic sections A-B and C-D. For locations see Fig. 1. Vertical scale in ms, two-way travel times below sea floor. R, rockhead; 1, surface of Formation 1; 2, surface of Formation 2.

the size and activity of the parent glaciers. Formation 3 sediments were deposited at a distance from the ice. When the supply of Formation 3 sediment became exhausted and sea level readjusted to its present position the deposits of Formation 4 were formed.

This study is being undertaken as part of the research programme of the Institute of Geological Sciences.

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Carbon fibres with large breaking strain

It has been predicted¹ that carbon fibre with a Young's modulus near 200 GN m⁻² could be prepared from acrylic precursors to yield elastic breaking strains of 2 to 3% with the proviso that an internally clean precursor was selected, judiciously processed and then surface treated to remove the more serious surface flaws. The prediction was made on the basis of gross internal and surface flaws controlling the strength characteristics of acrylic-based carbon fibres, at least in the region of carbonisation temperatures 1,000 to 1,300° C and in the associated modulus range 140 to 240 GN m⁻².

Fibres with such properties have now been prepared in experimental quantities. The breaking strain of carbonised (1,000° C) acrylics with no subsequent surface treatment is limited to about 1.0% in the modulus range 140 to 210 GN

TABLE 1 Single fibre properties of as-processed fibre; gauge length 23 mm

Cross-sectional area (10 ⁻¹² m ²)		Strength, (GN m ⁻²)		Young's modulus (GN m ⁻²)		Breaking strain (%)	
Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
48.1	5.9	3.17	0.30	207	10	1.53	0.20

m⁻² (ref. 2) and fibre processed to much higher temperatures inevitably yields lower breaking strain^{3,4}. Apart from an isolated report of a few ultra-high strength carbon fibres from Japan⁵, commercial fibres are being offered⁶ which have been surface-treated to give mean breaking strains just above 1.3%.

The acrylic previously shown to have a low internal flaw concentration of 10 flaws per metre (ref. 1) was processed through a batch oxidation, continuous carbonisation route to 1,100° C. The resultant fibre had single fibre properties quoted in Table 1.

TABLE 2 Frequency of fracture initiation by internal flaws¹².

Gauge length (mm)	Internal flaw fracture frequency $F_{int}/(F_{int} + F_{sur})$	Precursor internal flaw prediction $(1 - P)$
10	0.13	0.11
23	0.31	0.33
50	0.30	0.45

Because of the excellent control of processing parameters during preparation the Young's modulus was acceptable and the breaking strain already high. These fibres were submitted to an improvement of previously used surface treatments^{1,3,7,8} to modify the surface flaws normally operating. Treated fibres were then tested in tension by a rigorously controlled testing technique at gauge lengths of 10, 23 and 50 mm; at least 25 fibres were randomly selected for each gauge length. In addition a microscopic loop test⁹⁻¹¹ was carried out to estimate breaking strain at the very short effective gauge length of 0.1 mm. Because the loop test is particularly insensitive to well-spaced but severe flaws it provides some idea of the intrinsic breaking strain between severe stress-raising discontinuities.

Cross-sectional areas of the dog-bone-shaped fibres (Fig. 1) were determined by planimeter measurement of photographic enlargements of collected fractured ends at $\times 2,500$. This

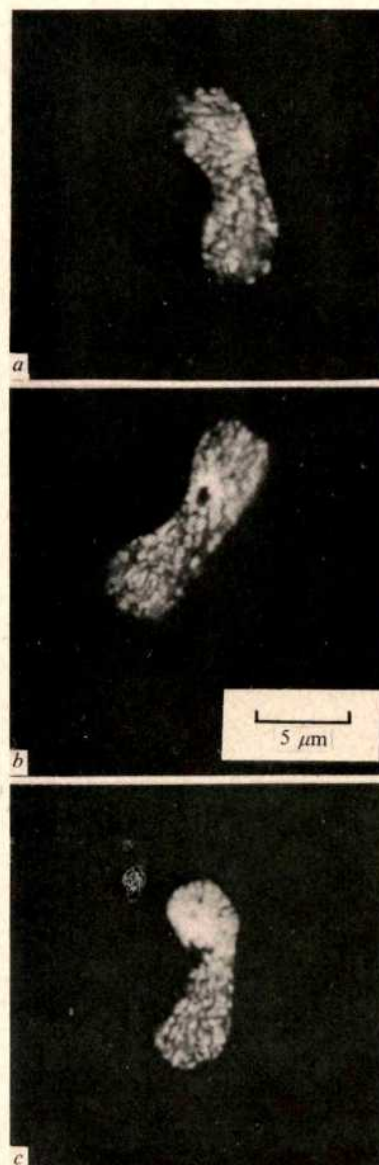


FIG. 1 Fracture surfaces of fibres with high breaking strain broken in tension ($L_g = 50$ mm), showing fracture initiated at: a, modified surface flaw ($\epsilon = 2.21\%$); b, internal flaw ($\epsilon = 1.78\%$); c, residual severe surface flaw ($\epsilon = 1.1\%$).

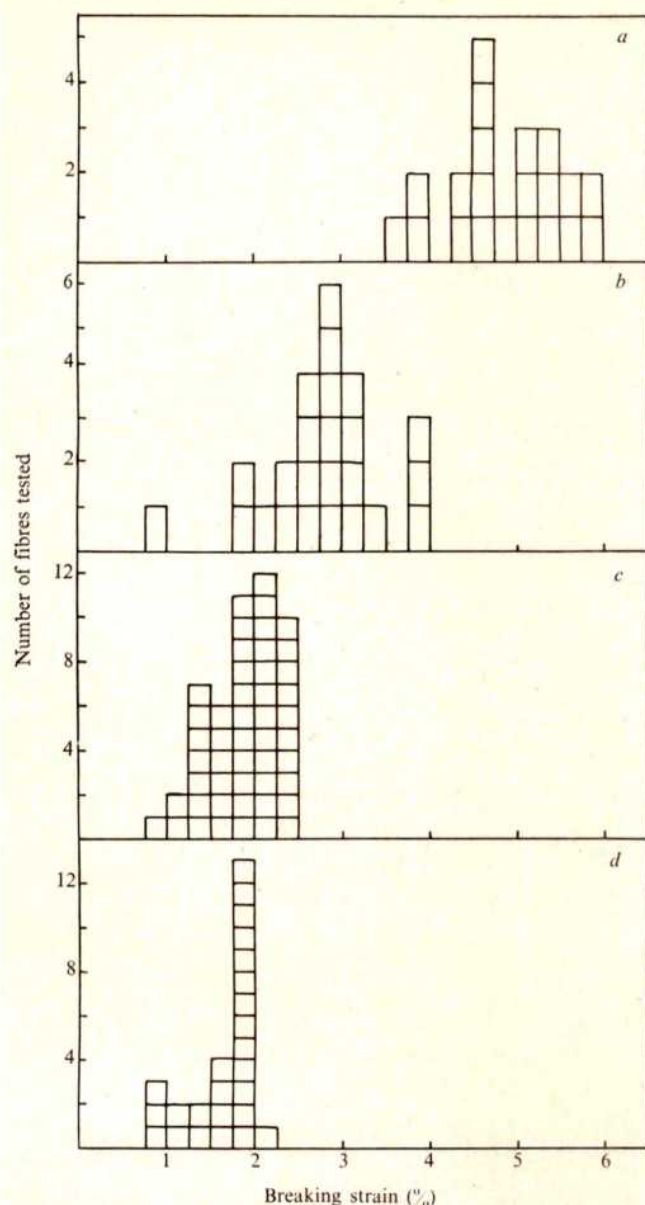


FIG. 2 Breaking strain distributions for various gauge lengths. a, Loop; b, $L_g = 10$ mm; c, $L_g = 23$ mm; d, $L_g = 50$ mm.

method has been cross checked for round fibres against normal optical measurement of fibre diameter. Although the probable error is still large at $\pm 7\%$, at least the area is determined at the point of fracture so it is likely to be more correct for fibre strength than for modulus (for which a mean cross-sectional area along the gauge length is required). The Young's modulus value has been verified by alternatively testing the fibres in an epoxide resin composite using standard composite techniques. Breaking strain determinations however, remain independent of fibre cross-sectional area and are favoured in the present discussion. Since the fibres behave essentially elastically to the point of failure, values of breaking strain adequately reflect the strength characteristics.

Examination of fractured ends of fibre, previously carried out by scanning electron microscopy^{1,3,7,12}, was carried out by high quality optical microscopy. The fracture pattern invariably indicated the fracture initiation source (Fig. 1). From fractography studies the frequency of fracture initiation by internal flaws¹ could be elucidated as well as breaking strain distribution associated with those internal flaws.

Breaking strain distribution data are given in Fig. 2 for various gauge lengths. There is a strong gauge length effect but mean breaking strains in excess of 2.0% are achieved at

gauge lengths less than 23 mm. Such strains will, it is believed, become operative in normal tensile loading of well bonded CFRP composites. It is also strikingly obvious that there has been a significant increase in the loop breaking strain ($\bar{\epsilon} = 4.9\%$) over the previously reported values of $\epsilon = 2.8\%$ (ref. 1) and $\bar{\epsilon} = 3.2\%$ (ref. 11). A typical loop at an apparent strain of 5.0% is shown in Fig. 3. At these high strains the loop was slightly distorted into a spiral. This can provide a significant underestimation of loop diameter in normal microscopic observation, correction for which was made by additionally measuring the height difference between sides of the loop at the point where loop diameter is measured.

Spiral distortion also introduces a deviation from elastica behaviour and $\epsilon = r/R$ (ref. 11); the loop strains are probably overestimated therefore but it can be safely assumed they are well in excess of 3.0%. This conclusion is supported by the significantly high proportion (33%) of fibres which had breaking strains in excess of 3.0% in the tensile tests at 10 mm gauge length (Fig. 2).

In normal tensile tests of this and other samples from the same acrylic precursor a significant number of fractures were initiated at internal flaws to provide the distribution of Fig. 4. These data were not available in previous studies¹ to adequately predict the gauge length effect of internal flaws. It was previously assumed that when internal flaws caused

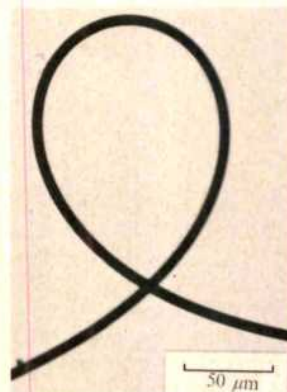


FIG. 3 Fibre sustaining a strain of 5.0% in loop test.

fracture the strength would be reduced on average to 0.33 of the intrinsic strength between flaws (loop test). It can now be seen for this particular acrylic-based fibre that the internal flaws are not very severe and operate in a comparatively narrow distribution about a mean breaking strain of 1.71% (mean strength 3.5 GN m^{-2}). If these internal flaws

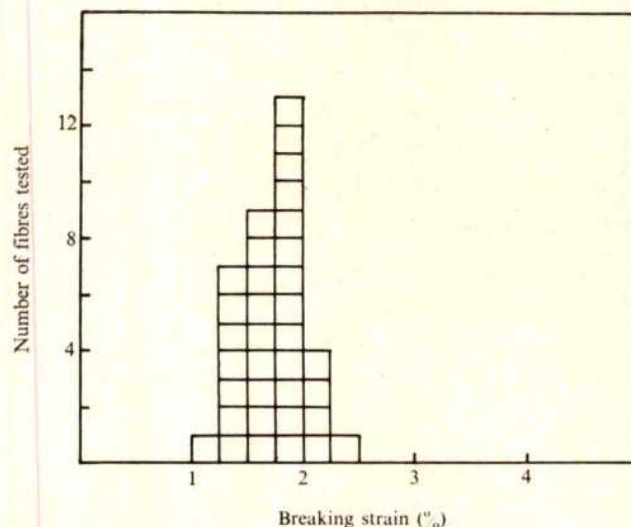


FIG. 4 Breaking strain distribution associated with fractures initiated at internal flaws.

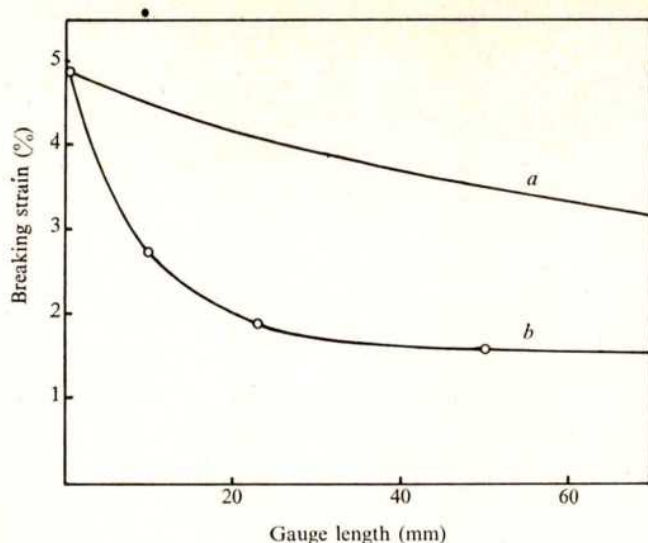


Fig. 5 Predicted (a) and experimental (b) mean breaking strain as functions of gauge length. Breaking strain standard errors are 0.13, 0.14, 0.06 and 0.07 for the four points on the curve in order of decreasing breaking strain.

are permitted to operate fully, then the gauge length effect can be predicted from their distribution in the original precursor acrylic¹ using

$$\epsilon = \epsilon_1 P + \epsilon_2 (1 - P)$$

where $\epsilon_1 = 4.9\%$ (loop tests), $\epsilon_2 = 1.71\%$ (Fig. 4) and P is the probability of avoiding an internal flaw for the particular gauge length^{1,13}. This is shown in Fig. 5, with the present experimental results. In spite of the improved breaking strains now achieved there is apparently a significant potential still to be tapped, even allowing for overestimation of loop breaking strain. The present shortfall in experimental values of breaking strain is partly explained by the persistence of some surface flaws causing fracture at low strain values, as indicated by the low strain tails of the distributions of Fig. 2. An example of such a fracture is illustrated in Fig. 1c. These particular surface flaws must be fairly well spaced at 50 to 100 mm and obviously contribute to the strong gauge length effect of Figs 2 and 5. In addition, they restrict the full operation of internal flaws, particularly at the longer gauge lengths. This is verified by the fracture frequency results of Table 2 where reasonable agreement between experimental and predicted frequencies is seen to extend only up to gauge lengths of 23 mm.

In addition to the achievement of breaking strains in excess of 2.0% at acceptable gauge lengths, the persistent operation of gross flaw mechanisms of fracture has been clearly demonstrated. There is no serious conflict between this treatment and the recent prediction of strength being dependent on ribbon wrinkling in graphitised fibre¹⁴. It has been recognised that structural features in addition to gross flaws may become progressively operative in controlling fracture in carbon fibre processed to graphitisation temperatures.

These studies confirm the earlier prediction on the feasibility of high breaking strain (2 to 3%) carbon fibres¹. They also form a satisfactory technical conclusion to the coherent and consistent treatment^{1,3,7,11-13} of carbon fibre strength and breaking strain on the basis of gross flaw mechanisms of brittle fracture applying at least to acrylic-based fibres processed to the carbonised stage. There remains scope for further development with the improvement of surface treatment techniques. The intrinsic breaking strain looks promisingly high, well in excess of 3.0%, and the strength correspondingly well above 6.0 GN m⁻² (0.9×10^6 pound inch⁻²).

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Crystal structure of polyvinyl alcohol

THE first comprehensive X-ray analysis of polyvinyl alcohol (PVOH) showed that the thirty observed reflections could be indexed according to a pseudo-orthorhombic cell¹. Bunn² later proposed a monoclinic unit cell comprising two molecules lattice parameters: $a = 7.81 \text{ \AA}$; $b = 2.52 \text{ \AA}$; $c = 5.51 \text{ \AA}$; $\beta = 91^\circ 42'$.

It was thought that the periodicity of 2.52 \AA must imply an isotactic configuration with hydroxyl groups in identical positions along the backbone chain. This rather unlikely

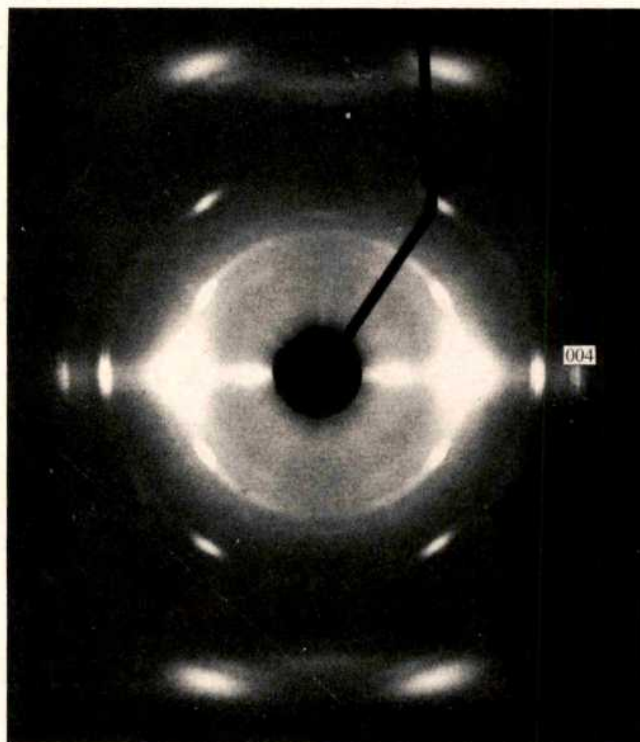


Fig. 1 X-ray diffraction photograph of crystalline PVOH fibre. 2θ for the 004 reflection is $33^\circ 40'$.

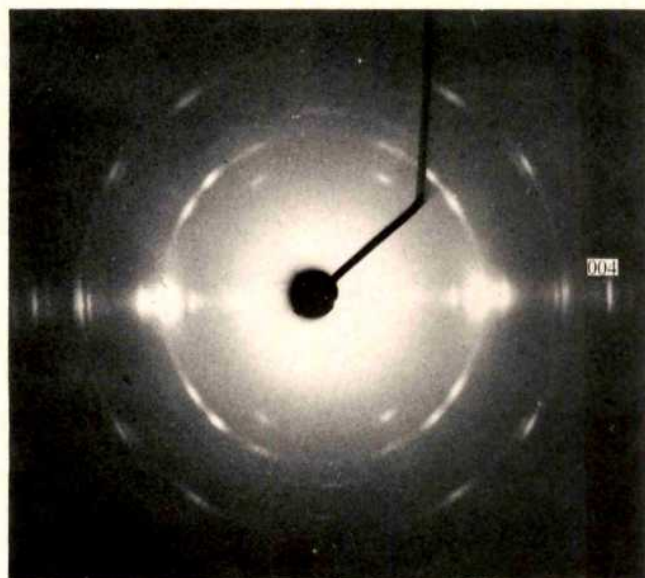


FIG. 2 X-ray diffraction photograph showing both crystal forms. 2θ for the 004 reflection is $33^\circ 40'$.

structure was modified by Bunn, who postulated that the repeat distance of 2.52 \AA was "equally compatible with a molecular structure in which hydroxyl groups are randomly placed in left and right-handed positions".

Despite several later analyses³⁻⁵ the same basic lattice parameters and molecular configuration are widely accepted today. Mochizuki⁵ quoted a slightly higher fibre identity period of 2.533 \AA which he obtained from a tilted-fibre photograph and, in agreement with Bunn, he considered PVOH to have a planar zigzag configuration. From an azimuthal scan of the so-called 100, 200 and 101 reflections, he found that as well as a maximum on the equator of the diffraction pattern, there was another at about 30° from the meridional axis. He therefore postulated that not all of the crystallites in an oriented fibre tend to arrange themselves with their long axis parallel to the fibre axis. Some are tilted by a very considerable angle (about 60°) to the direction of draw. No possible reason was given for this.

One of the most interesting problems encountered in the X-ray analysis of PVOH concerns the molecular conformation of stereoregular polymer. Both syndiotactic-rich and isotactic-rich PVOH have been prepared from the corresponding formate⁶. It is rather surprising, however, that no essential differences were noticed between their X-ray diffraction patterns—each showed an identity period of 2.5 \AA .

I have found that the crystal structure of PVOH is somewhat more complex than has been previously realised. I used a commercial PVOH (Elvanol 71-30) to prepare highly drawn fibres because the chemical characteristics are advantageous for fibre production⁷. The more important qualities include a very high degree of hydrolysis (99%–99.8%), a suitable molecular weight (80,000) and a maximum ash content of 1%. A 20% aqueous solution of this polymer was extruded into a coagulation bath containing concentrated ammonium sulphate solution. No appreciable tension was applied at this

TABLE 1 Lattice parameters of the two crystal structures of PVOH

α form (monoclinic)	β form (orthorhombic)
$a = 15.58 \text{ \AA} \pm 0.02 \text{ \AA}$	$a = 15.86 \text{ \AA} \pm 0.03 \text{ \AA}$
$b = 7.59 \text{ \AA} \pm 0.01 \text{ \AA}$	$b = 5.95 \text{ \AA} \pm 0.02 \text{ \AA}$
$c = 10.65 \text{ \AA} \pm 0.02 \text{ \AA}$	$c = 10.54 \text{ \AA} \pm 0.03 \text{ \AA}$
$\beta = 96^\circ 00' \pm 10'$	

TABLE 2 Observed and calculated values of $\sin^2 \theta$ for the monoclinic cell

Index	Intensity	$\sin^2 \theta$ observed	$\sin^2 \theta$ calculated
200	m	0.009	0.009
002	m	0.021	0.021
202	vs	0.028	0.029
012/310	vs	0.032	0.031/0.032
400	m	0.039	0.039
020	m	0.041	0.041
203	s	0.060	0.059
022	vs	0.063	0.062
402	s	0.063	0.063
502	w	0.077	0.078
313	w	0.083	0.083
004	s	0.084	0.084
030	s	0.093	0.093
314	m	0.113	0.112
323	s	0.112	0.114
331	m	0.122	0.121
612	m	0.123	0.124
024	s	0.125	0.125
622/523	m	0.154	0.155
800	w	0.154	0.157
315	w	0.159	0.158
810	w	0.165	0.167
006	m	0.188	0.189
106	m	0.194	0.193
334	w	0.203	0.203
515/911	w	0.210	0.211
026/126	w	0.231	0.230/0.231
335/732	w	0.242	0.241
007	m	0.254	0.257
705	m	0.264	0.263
207	m	0.271	0.272
1200	w	0.351	0.353
060	m	0.372	0.372
906	m	0.406	0.406

w, weak; m, medium; s, strong; vs, very strong.

stage. After complete coagulation, a wet-drawing stage was incorporated in order to impart a degree of orientation sufficient to prevent dissolution of the fibres at the washing stage. After being collected and dried, the product was hot-drawn over a plate maintained at 200°C . Cylindrical and flat-film X-ray diffraction photographs were taken, using nickel-filtered $\text{CuK}\alpha$ radiation with exposure times from 1.5 to 4 h. Finely powdered copper was used as an internal standard, and the spacing of 2.088 \AA was chosen as the reference line (American Society for Testing Materials, Powder Data File No. 4-0836).

X-ray diffraction photographs show many more reflections than have been previously noticed (Fig. 1). Of particular importance are those lying between the equator and the meridian, because they immediately indicate the existence of a somewhat larger unit cell than has been previously recognised. A detailed study of the diffraction pattern shows that no single unit cell can adequately account for all of the observed reflections. A system embodying two crystal structures will, however, account for them very satisfactorily. I therefore propose that polyvinyl alcohol crystallises in two forms which have the lattice parameters presented in Table 1. Tables 2 and 3 compare the observed and calculated values of $\sin^2 \theta$ based on these cells. The agreement is very good. Calculated density values agree with observed values for highly crystalline fibres. Assuming 18 monomers per orthorhombic unit cell, a density of 1.322 g cm^{-3} is obtained. For the monoclinic system, a density of 1.400 g cm^{-3} is obtained, assuming 24 monomers per cell.

Generally speaking, reflections belonging to the orthorhombic system are weak in intensity, presumably as a result of a structural preference for the monoclinic system. By altering both the total draw ratio and the temperature of the hotplate, fibre samples having varying proportions of the two crystal structures can be prepared. Figure 2, for ex-

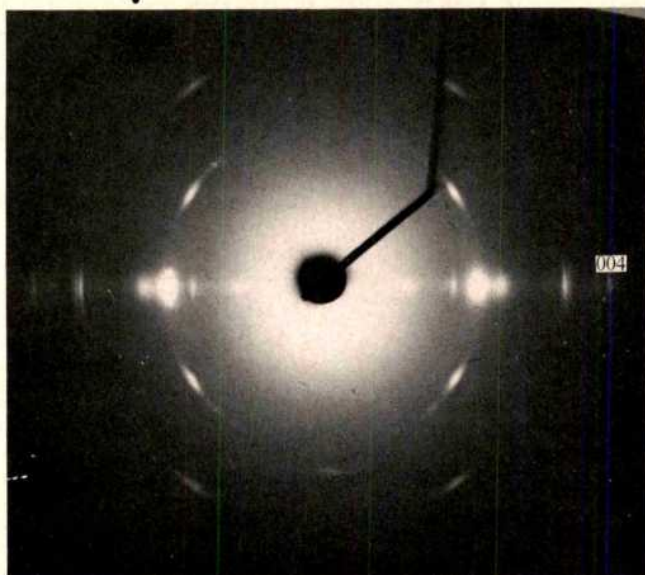


Fig. 3 X-ray diffraction photograph showing the monoclinic form only. 2θ for the 004 reflection is $33^\circ 40'$.

ample, shows reflections of up to 35° (2θ values) and exhibits both crystal forms. On the other hand, Fig. 3, consists only of reflections from the structure rich in the monoclinic form. Although the precise configuration of the polymer chains within the crystalline regions is unresolved at present, the fibre periodicities suggest a twisted or helical structure for both crystal forms. The monoclinic system, with a periodicity of 7.59 Å, may be explained in two ways. It is possible that the polymer exists as a kinked chain in which every third hydroxyl group is in an equivalent position. Alternatively, if there is a high degree of syndiotacticity, a structure incorporating four monomer units per fibre repeat may be present. This latter type of configuration has been claimed for syndiotactic polypropylene⁸ and has been suggested for polyacry-

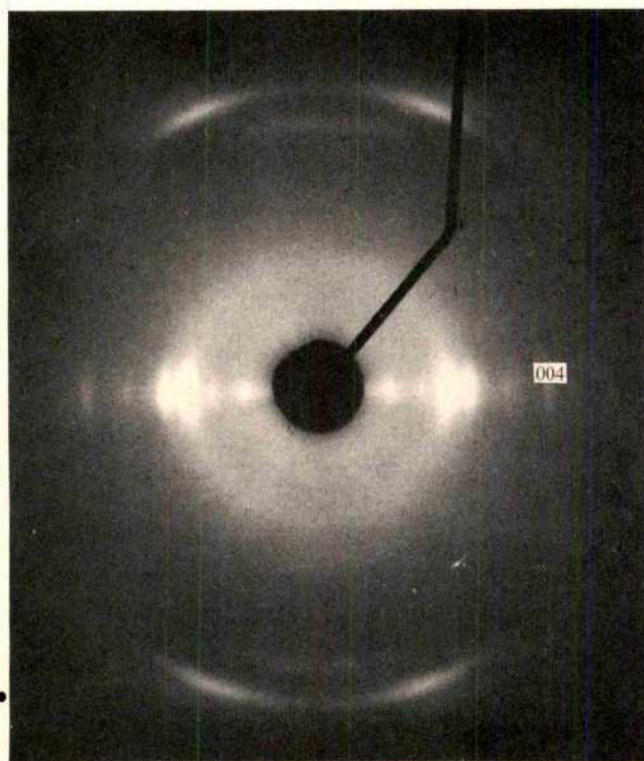


Fig. 4 Effect of water on the diffraction pattern of PVOH. 2θ for the 004 reflection is $33^\circ 40'$.

TABLE 3 Observed and calculated values of $\sin^2 \theta$ for the orthorhombic cell

Index	Intensity	\sin^2 observed	\sin^2 calculated
011	m	0.022	0.022
202	s	0.030	0.031
211	m	0.031	0.031
203	w	0.056	0.057
312	w	0.059	0.059
013	w	0.064	0.065
601	w	0.094	0.090
322	w	0.108	0.110
404	w	0.122	0.124
223	w	0.122	0.125
030	w	0.155	0.151
426	w	0.293	0.300

w, weak; m, medium; s, strong.

lonitrile (B. G. Colvin, and P. Storr, not yet published). These polymers have identity periods of 7.4 Å and 7.1 Å respectively. Furthermore, it is well known that the infra-red spectrum of PVOH shows a fairly high absorbance at 916 cm^{-1} , a wave number usually associated with the syndiotactic form. I feel, however, that the former proposal is in fact the more likely, because the 030 and 060 reflections are relatively strong and the 020 and 040 reflections are not visible at all (Table 1). It therefore seems that crystalline PVOH consists of a kinked chain with three monomers per repeat. The distortion of the main chain is regular and is apparently governed by a combination of mutual repulsion between neighbouring hydroxyl groups and a complex hydrogen-bonding mechanism.

It is reasonable to suppose that stereoregular PVOH may be examined more successfully provided that polymer samples of high crystalline order can be obtained. The following parameters are found to have a pronounced effect on the amount of detail manifested in a wide-angle X-ray diffraction pattern. First, the degree of jet-stretch is of some importance. This should be kept to a minimum, as it has been observed that a higher crystalline order is obtained by allowing the fibre to relax as much as possible in the coagulation bath. Second, a high degree of orientation is of the utmost importance, and this should be imparted principally at the wet-drawing stage. Third, dilute solutions of coagulant salts should be avoided as much as is practically possible. Water molecules adversely effect the naturally occurring hydrogen-bonding mechanism which is responsible for high degrees of order (Fig. 4), and washing stages should therefore be kept to a minimum. Even after crystallisation has occurred to a very high degree (Fig. 1), water molecules can penetrate the crystalline regions and thus modify the molecular packing arrangement. The only noticeable periodicity in the fibre direction after such treatment, results from the chemical repeat of 2.5 Å. Presumably the kinked chain is no longer regular as a result of the disruption of hydrogen bonds within the polymer network. Crystallisation can still occur, however, but to a much lesser degree. The small size of the hydroxyl group is probably the reason that PVOH can crystallise at all under these conditions². Whether there is any marked variation in the lateral packing of chains is uncertain at present, although no pronounced variations in the equatorial reflections have been noticed. It is proposed that more information may become available from fibres of varying tacticity produced in the near absence of water. The use of organic precipitating baths may prove useful in this respect.

This analysis also suggests that there is now no real evidence to support a 'preferred tilted-crystal orientation' theory, because an azimuthal scan of the so-called 100, 200 and 101 reflections would necessarily give rise to other maxima. These, however, arise from quite separate reflections, namely the 012 or 310 of the monoclinic system and the 211 of the

orthorhombic cell. It must be presumed that Mochizuki⁵ had prepared a sample of PVOH showing some signs of increased crystalline order but, instead of assigning separate indices to the off-equatorial maxima, he included them as part of the equatorial arcs.

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Estimation of solubility parameters of nonelectrolytes

THE Hildebrand approach^{1,2} provides an explanation of the solubility of nonelectrolytes based on a thermodynamic method. In this method used for the estimation of solubility, it is assumed that each molecule possesses a characteristic potential energy based on intermolecular forces which may be described as the cohesive energy density. The latter may be defined as the potential energy of 1 cm³ of liquid or the energy required to separate all molecules in a unit volume of liquid. This value is zero when the molecules are randomly dispersed in a solution.

The solution process may be compared with the vaporisation process in which molecules at an equilibrium distance are transported to an infinite distance. Accordingly, the energy of vaporisation is used to quantify the solution process in the Hildebrand approach. Thus, the solubility parameter which is represented by the symbol δ is equal to the square root of the cohesive energy density (CED). The latter is equal to $\Delta E_v/V$ in which ΔE_v is the energy of vaporisation and V is the molar volume.

As shown by the following equations, values for the solubility parameter (δ) which is the square root of CED, may be calculated from the heat of vaporisation per mole (ΔH_v), the gas constant (R), the temperature on the Kelvin scale (T) and the molar volume (V):

$$\begin{aligned}\delta &= (\text{CED})^{1/2} = \left(\frac{\Delta E_v}{V}\right)^{1/2} = \left(\frac{\Delta H_v - RT}{V}\right)^{1/2} \\ &= \left(\frac{\Delta H_v - RT}{M/D}\right)^{1/2} = \left(\frac{D(\Delta H_v - RT)}{M}\right)^{1/2}\end{aligned}$$

The above equation is derived from the following relationships:

$$\Delta H_v = \Delta E_v + P\Delta V = \Delta E_v + RT \therefore \Delta E_v = \Delta H_v - RT$$

$$D = M/V \therefore V = M/D$$

Because of the difficulty in obtaining values for the molar volume, the quotient of the gram formula weight (M) and density (D) is substituted for the value V . The units for δ values obtained from the preceding equation are (calorie cm⁻³)^{1/2} but the term hildebrand has been adopted in some instances for this unit³. According to the solubility parameter concept, solubility occurs when the δ values of solid solute and a liquid solvent are within a range of 2 hildebrands (2 H).

The quality of hydrocarbon solvents such as benzene, cyclohexane or hexane is readily described by δ values and these nonpolar solvents are called regular solvents. But the potential energy of solvents increases with polarity because of additional interactions resulting from dipole-dipole (Keesom) forces, induced dipole-dipole (Debye) forces and acid-base type (Lewis) forces and provisions must be made to account for these additional interactions in applications of the solubility parameter concept. Burrell⁴ has classified solvents as poor, moderately bonded and strongly bonded solvents.

Several graphical approaches have been suggested for the estimation of δ values. Values of ΔH at 298 K may be estimated from the boiling points (T_b) and these values may be used for the calculation of δ values by use of the following equation⁴:

$$\Delta H_{298} = 23.7T_b + 0.02T_b^2 - 2,950$$

(see Fig. 1).

It has also been shown previously⁵ that the contribution of the methylene group (CH₂) to the δ values is constant⁵ and so the δ values for solvents in a homologous series may be estimated from the graph shown in Fig. 2 (ref. 6).

The logarithm of δ values of nonpolar solvents such as hexane ($\delta = 7.3$), cyclohexane ($\delta = 8.2$) and benzene ($\delta = 9.2$) are related to readily obtainable physical constants as shown by the following equation and by line A in Fig. 3;

$$\log \delta \simeq 0.50 \log \frac{T_b D}{M} + 0.655$$

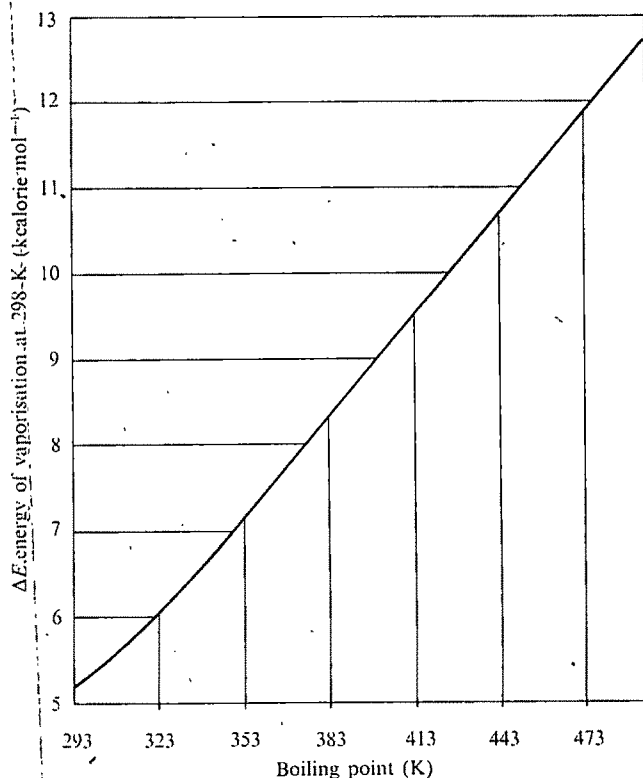


Fig. 1 Relationship of ΔE_v and boiling point.

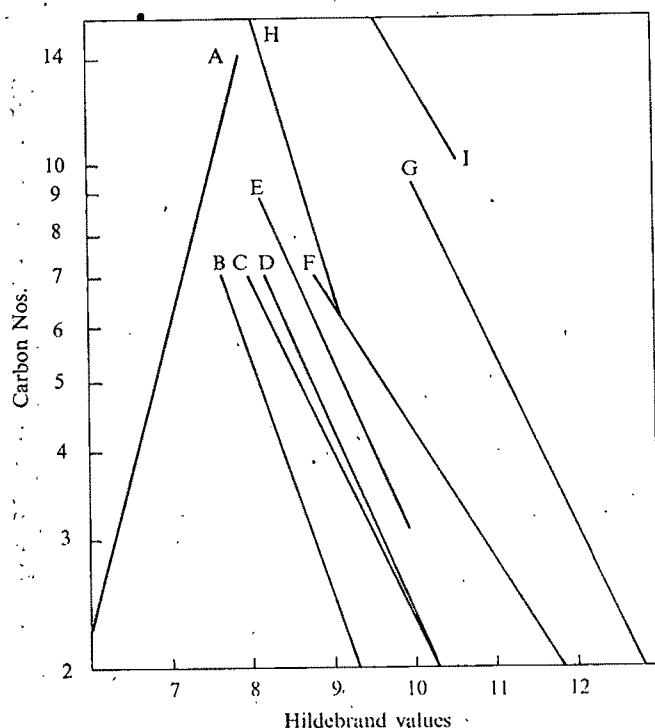


Fig. 2 Relationship of solubility parameters and carbon numbers in various homologous series of solvents. A, Normal alkanes; B, normal chloroalkanes; C, methyl esters; D, alkyl formates and acetates; E, methyl ketones; F, alkyl nitriles; G, normal alkanols; H, alkyl benzenes; I, dialkyl phthalates.

A slightly different equation may be used to estimate the logarithm of δ values for moderately bonded solvents as shown below:

$$\log \delta \cong 0.40 \log \frac{T_b D}{M} + 0.665$$

These values which apply for solvents such as ethyl ether ($\delta = 7.4$), butyl acetate ($\delta = 8.0$), acetone ($\delta = 9.9$) and dimethyl sulphoxide ($\delta = 12.0$) are shown as line B in Fig. 3.

The logarithm of δ values of strongly hydrogen bonded solvents may be estimated from line C in Fig. 3 or from:

$$\log \delta \cong 0.40 \log \frac{T_b D}{M}$$

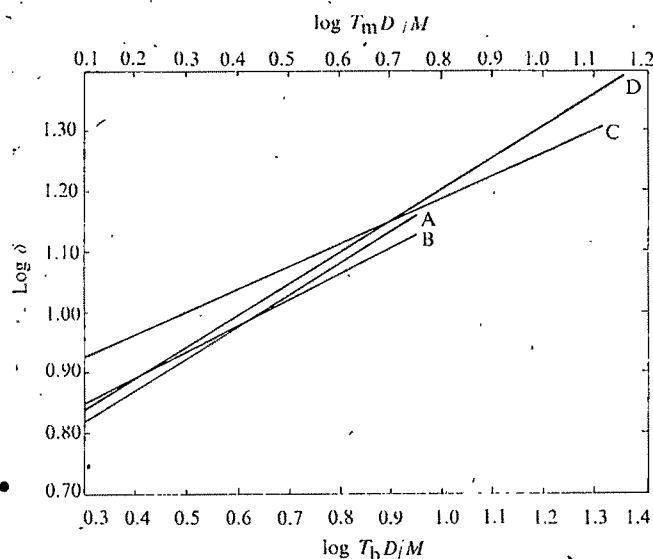


Fig. 3 Relationship of $\log \delta$ and \log of readily available constants.

Typical strongly hydrogen bonded solvents are diethylamine ($\delta = 8.0$), sec. amyl alcohol ($\delta = 10.0$), acetic acid ($\delta = 10.1$), and aniline ($\delta = 10.3$). Because they exist primarily as dimers, the M values used for formic and acetic acids are twice the molecular weights of the simple molecules.

The logarithm of δ values of solids such as naphthalene ($\delta = 9.9$), β -bromonaphthalene ($\delta = 10.6$) and maleic anhydride ($\delta = 13.6$) may be estimated from line D in Fig. 3 and from:

$$\log \delta \cong 0.50 \log \frac{T_m D}{M} + 0.670$$

Tables of solubility parameter values are readily available⁴. As shown by the following example, solubility parameters may also be readily calculated from the graphs included in this report. The boiling point of carbon tetrachloride is 77°C or 350 K. According to Fig. 1, the value of ΔE for carbon tetrachloride is 7.2 kcal/mol. Therefore, by use of the relationship

$$\delta = \left(\frac{\Delta E}{V} \right)^{1/2} = \left(\frac{D(\Delta E)}{M} \right)^{1/2} = \left(\frac{7,200(1.6)}{154} \right)^{1/2}$$

one obtains a δ value of about 8.4 H for carbon tetrachloride.

Similarly, according to line H in Fig. 2, the δ value for toluene ($C = 7$) would be about 8.9. The scale for the ordinate is an arbitrary one chosen so that linear relationships could be demonstrated when the number of carbon atoms in the solvent was plotted against the solubility parameter values in hildebrand units. The δ value of a strongly hydrogen bonded solvent such as methanol may be estimated from line C in Fig. 3 by using the values $T_b = 338$ K, $D = 0.79$ g/cm³, $M = 32$. So

$$\log \frac{T_b D}{M} = 0.922$$

Thus, $\log \delta = 1.16$ and $\delta = 14.5$ H.

In addition to providing quantitative values in place of the qualitative expression, 'like dissolves like,' the solubility parameter may be used to explain that while cellulose nitrate ($\delta = 10.5$ H) is insoluble in either ethanol ($\delta = 12.7$ H) or ethyl ether ($\delta = 7.94$), it is readily soluble in an equimolar mixture of these two solvents ($\delta = 10.0$ H). This solution, called collodion or newskin, is still in use today.

The solubility parameter may also be used to explain many well known unique solvent systems such as a saturated aqueous solution of urea. This solution of natural origin has been used to dissolve dried blood, to soften animal skins, to set hair, to disperse the ingredients of gun powder, to aid the inking of lithographic plates, to make wood flexible and to improve the flow of cold house paints.

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Applications of Boolean programming optimisation methodology to the direct solution of crystal structures

We have found that Boolean optimisation methods can be used to derive phase information from observed X-ray structure factors. This will provide a new approach to the problem of the loss of phase information during X-ray diffraction experiments, which has been a major limitation to the study of the structure of matter. The possibilities of the method are demonstrated here in the successful application of integer programming optimisation techniques to the three-dimensional solution of centrosymmetric structures.

Current direct methods¹⁻⁵ involve statistical relationships among a group of reflexions preselected to be strongly influenced by the atomic arrangement. The phase problem assumes two levels of complexity depending on (1) the absence or (2) the presence of a centre of symmetry. (1) In the more complex general case, the phase angle is free to assume all values from 0° to 360°. (2) The presence of a crystallographic centre of symmetry constrains the phase angle to 0° or 180°, and the cosine of the phase angle is therefore constrained to ± 1 .

The phase relationships can be formulated in terms of classical integer programming. For the centrosymmetric case, the variable, X , equal to zero or one, corresponds to a sign or $-$ or $+$ (for example, $\text{sign} = -(-1)^X$). Freeman⁶ and Dakin⁷ have explored the use of integer programming techniques in the one-dimensional case, and Lesk⁸ redetermined the planar structure of dihydrouacil in a two-dimensional integer programming formulation. We now report the application of integer programming techniques in three dimensions to the solution of the hitherto unstudied molecule, spiro-[5.5]-undeca-1,4,7,10-tetraene-3,9-dione⁹, 'spirodienone'. We have also redetermined the structure of potassium lead hexanitrate(II), $\text{K}_2\text{PbCu}(\text{NO}_3)_6$, using the structure factors of Cullen and Lingafelter¹⁰.

We have found that certain integer programming methods fail when identical constraints are used. Therefore, a disjoint set of inequality constraints was chosen to place upper and lower bounds on the values of electron density at points in the crystallographic unit cell. For the spirodienone study, choice of these points was assisted by a procedure similar to the application of the symmetry minimum function to Patterson(vector) space¹¹. Pronounced valleys are located on the map of the Patterson function and a valley in vector space is related to positions in real space where no atom is expected to appear. For the centrosymmetric case, a point of minimum density in vector space at $2x, 2y, 2z$ can be mapped into real space at x, y, z , together with the related points within the unit cell generated by symmetry and translations. For space group $\text{P}\bar{1}$, points need to be mapped into only half the unit cell, which is the asymmetric unit. In higher symmetry space groups, the location of local minima in Harker lines and sections would help establish positions in real space for the assignment of constraints.

A typical constraint in space group $\text{P}\bar{1}$ can be constructed by setting bounds to the inequalities:

$$\sum_{j=1}^n A_{ij}X_j - b_i \leq \text{upper bound and}$$

$$\sum_{j=1}^n A_{ij}X_j - b_i \geq \text{lower bound.}$$

The index, i , refers to a point in real space; j refers to a reflexion. The variable X_j assumes binary values zero or one as the phase of reflexion j is assigned a value of 180° or 0°. The coefficient A_{ij} represents twice the value of the computed electron density at point i from structure factor j , (assuming a positive sign for each F value) that is $A_{ij} = (4/V) |F_j| \cos 2\pi(h_jx_i + k_jy_i + l_jz_i)$, where V is the volume of the unit cell. The term b_i is evaluated to be half the sum of all the terms A_{ij} at point i for n reflexions: that is, $b_i = 1/2 \sum_{j=1}^n A_{ij}$. The upper and lower bounds are

near-zero-valued parameters which define a window within the computed electron density summation must fit. The assignment of a value of one to phasing term X_j has the net effect of adjusting the electron density by the contribution of the positive structure factor j at that point. Conversely, if X_j is equal to zero, the effect is to adjust the contribution of reflexion j to the electron density due to the negative sign.

If the choice of variables X_j corresponded with the correct set of signs, the left-hand side of the inequality constraints would approximate the actual electron density at each point where the constraint is computed. Deviations from the actual electron density may be attributed both to errors inherent in the data set (which would usually be minimal) as well as to the incomplete nature of the data set due to limitations in computer size. When the objective function and a set of constraints are input to the surrogate constraint Zero-One Algorithm¹² then all feasible sets of phases which are found on the way to the optimum solution are output. For spirodienone, the coefficients in the objective function were biased by the computed value of the electron density due to each reflexion in the Fourier summation at the starting position. Then optimisation techniques 'tour' solution space in search of the optimum phases.

For the first trial of the method we chose spirodienone because of its structural interests to spectroscopists and its suitability for our procedure. Crystals of spirodienone, $\text{C}_{11}\text{H}_8\text{O}_2$, were grown from a dioxane solution of pure compound provided by Dr Guy Farges. Cell parameters are:

$$\begin{array}{lll} a = 9.08 \text{ \AA} & b = 13.61 \text{ \AA} & c = 7.49 \text{ \AA} \\ \alpha = 91.11^\circ & \beta = 96.91^\circ & \gamma = 100.50^\circ \end{array}$$

$\text{P}\bar{1}$, $Z = 4$. Thus there are two independent molecules of 13 non-hydrogen atoms in the asymmetric unit. The diffractometer data were reduced to normalised structure factors (E values). An analysis of the E values showed the presence of a strong subcell, therefore the structure was first solved by a direct methods program, MULTAN⁵, to establish that a solution would be forthcoming.

The method worked well when it was provided with the starting position of a single atom (taken from a peak of the MULTAN E map) to define the origin of the unit cell. The coefficients in the objective function were biased by the computed value of electron density due to each reflexion in the Fourier summation at that known position. For 170 variables (signs) and 160 constraints a near optimum solution was found within 80s on a CDC 6600 computer. The resulting 170 signed E values were input to a Fourier program and the resulting E map clearly indicated twenty-three of the twenty-six non-hydrogen peaks. Four cycles of full matrix least squares refinement of the twenty-three peaks produced an R factor of 30.0% (based on F) and a new set of signs that, in the next Fourier calculation, yielded the remaining three atoms. All non-hydrogen atoms together with their isotropic temperature factors refined to an R factor of 13.9%. Further refinement has lowered R to 4.8%.

We were not satisfied that *a priori* information would be a necessary condition for the proper functioning of the

optimisation approach. We therefore redetermined a known structure, this time in the cubic crystal class, with several heavy atoms (Pb, K, Cu) as well as light atoms (N, O) in the molecule. Potassium lead hexanitrocuprate (II) crystallises in space group $Fm\bar{3}$, $a_0 = 10.672\text{\AA}$.

Normalised structure factors were generated from the observed reflexions. The statistical distribution of the E values was highly uncharacteristic for both centric and acentric cases. The highest E was only 1.85. The application of the optimisation approach produced the correct structure in a straightforward manner.

The fifteen atomic parameters were sought by applying ninety lower-bound constraints on electron density. All points were chosen from an 0.5\AA grid in the asymmetric unit without reference to the Patterson map, as in the first structure. Fifty structure factors corresponding to the highest thirty-five E values in the parity group *ggg* and the highest fifteen in the other parity group, *uuu*, were used. The signs thus determined were used in a Fourier summation which yielded the correct structure. This second solution thus demonstrates that Boolean optimisation methods can derive phase information directly from the observed structure factors without *a priori* information. As these two analyses are our first two attempts to test the strengths and limitations of this approach, the results are encouraging. However, more study is required to define the critical parameters of the constraint's expression and the limitations of the method itself.

In conclusion, two analyses of the phase problem in the centrosymmetric case have been made. In the first trial, one atom at a known position was required to bring about phasing convergence. Although as mentioned, *a priori* information was required, this trial suggests the possibilities of this method for phase refinement or phase extension. As a second trial we chose a molecule in the cubic class. The method was able to proceed directly from structure factors to signs without *a priori* assumptions or information. Thus, a nonstatistical approach to direct methods is possible by means of Boolean programming optimisation methods.

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BIOLOGICAL SCIENCES

Premature ageing and occurrence of altered enzyme in Werner's syndrome fibroblasts

HUMAN diploid fibroblasts cannot be propagated indefinitely in culture¹. Longevity experiments with fibroblasts grown from skin biopsies from individuals of different age show that the greater the age of the donor, the shorter the lifespan of their cells in culture²⁻⁴. Patients suffering from Werner's syndrome age prematurely and have an average lifespan of 46 yr (ref. 5). Martin, Epstein and Sprague⁴ established primary fibroblast cultures from skin specimens from four such patients and discovered that these could be subcultured only 4-11 times before growth ceased. This compares with an average lifespan of 32 subcultures for skin fibroblasts from normal individuals in the same decades of life⁴. We show here that changes which occur in the enzyme glucose-6-phosphate dehydrogenase (G6PD) during the senescence of normal fibroblasts⁶, also occur during the premature senescence of cells from a patient with Werner's syndrome.

Werner's syndrome is an inherited condition, caused by a rare autosomal recessive allele⁵. A review and critical appraisal of the similarities between normal ageing and premature ageing in these patients is available⁵. The features of Werner's syndrome are first seen soon after adolescence. Normal development is then interrupted by greying of the hair and sometimes baldness, impairment of further bodily growth, loss of subcutaneous tissue and muscle, and progressive changes in the skin, all of which give the affected individual a prematurely aged appearance. Other changes which typically take place include bilateral cataracts, weakness of the voice and underdevelopment of the genitalia with consequent low level of fertility. Arteriosclerosis becomes manifest and calcium is deposited in arterial walls. The development of mild diabetes is common, and there is also an unusually high incidence of neoplasms. The combination

TABLE 1 Estimates of the heat-labile fraction of G6PD in Werner's syndrome cells, controls and MRC-5 cultures of different age.

Culture	Donor	Passage No.	% Heat-labile enzyme
Werner's skin fibroblasts	Male, age 46	7	21
		9	23
		9	22
		11	22
		11	21
Normal skin	Male, age 54	11	9
		25	8
		27	6
	Female, age 40	8	4
		22	4
Normal MRC-5	Male	10-40	range: 0-10
foetal lung fibroblasts		(27 experiments)	mean: 5.8
Senescent MRC-5	Male	53-68	range: 10-27
		(34 experiments)	mean: 16.7

Each determination is based on an experiment of the type shown in Fig. 1. The summary of results with MRC-5 are taken from Holliday and Tarrant⁶.

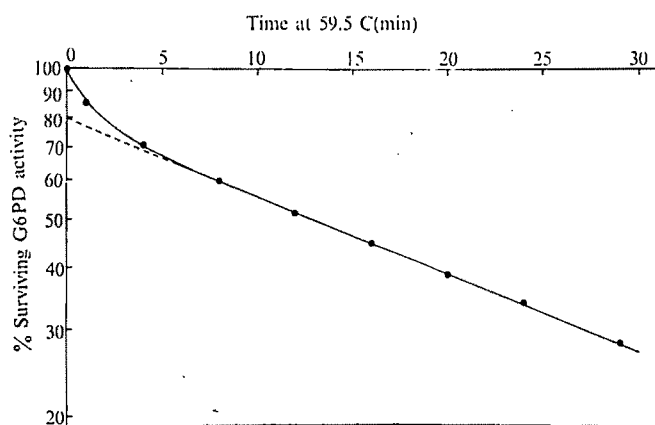


FIG. 1 The inactivation by heat of G6PD in a cell-free extract from passage 11 skin fibroblasts, derived from a patient with Werner's syndrome. The methods for the preparation of extracts and the assay of the enzyme are as previously described⁶.

of subcutaneous wasting with taut and shiny skin results in spindly extremities, which contrast with a stocky trunk. Callosities and later ulcers develop on the ankles as well as over the Achilles tendons of the heels. Although these ulcers are particularly indolent and resistant to effective treatment, vascular insufficiency does not seem to be responsible. Their failure to heal probably reflects the limited division potential of cells which normally replace tissue over these areas which are vulnerable to pressure and damage.

Several of these features in a male patient (DD), aged 40, led to the diagnosis of Werner's syndrome in 1966 (ref. 7). In July 1972 specimens of skin and subcutaneous tissue were taken from the forearm and samples were used to establish primary fibroblast cultures. Small pieces of tissue were placed under coverslips in Leighton tubes and incubated at 37° C in Eagle's basal medium containing 10% foetal calf serum and 10% tryptose phosphate broth. Half the medium was changed twice weekly. When sufficient cells had migrated from the tissue and grown to form a monolayer, they were removed after trypsin treatment and subcultured into small glass bottles. In some cases several populations of cells could be collected at intervals of 2-3 weeks from the same Leighton tube. The cells were passaged with a 1:2 split ratio using the standard procedures for fibroblast cultures⁸, although growth of the Werner's cells was considerably slower than with normal fibroblasts. Of the various cultures established, none survived beyond passage 12. This is in agreement with the results obtained by Martin *et al.*⁴.

Sufficient cells were obtained to carry out several experiments with G6PD, but there was insufficient material to examine any other enzyme. It has previously been shown that healthy cultures of foetal lung strain MRC-5 contain on average about 5% of altered G6PD which is heat labile, whereas senescent cultures can obtain up to 27%, with an average of about 17%⁶. Although it is not ruled out that this altered enzyme is produced by post-synthetic modification, the overall evidence indicates that the errors in protein synthesis are responsible for the changes in the enzyme which are seen during ageing⁶. Five experiments were carried out with cell-free extracts from cultures of Werner's cells, and in each case about 20% heat-labile enzyme was detected. One of these experiments is shown in Fig. 1, and the overall results are given in Table 1. The estimate of the proportion of altered G6PD is accurate to within about 3%. We have examined G6PD from cultured fibroblasts from three normal adults. In each case the amount of altered G6PD is in the same range as non-senescent MRC-5 cultures. These results are given in Table 1. A mixture of a cell-free extract from Werner's cells (21% heat-labile enzyme) and an equal amount of control enzyme (9% heat-labile) gave

the expected intermediate level of altered G6PD (16% heat-labile).

Our results with G6PD show that cells from Werner's patients have at least one biochemical defect in common with senescent fibroblasts derived from a normal individual, and this strengthens the view that the early death of Werner's cells in culture is really due to premature ageing, and makes it more likely that there is a direct relationship between *in vivo* and *in vitro* ageing. The results also add weight to the growing evidence that ageing is associated with the production of defective proteins^{8,9-14}, although so far there is little to indicate whether this is a primary cause of ageing or a secondary consequence¹⁵. One possibility is that the altered proteins arise from an ever increasing level of errors in protein synthesis, as originally suggested by Orgel¹⁶. A genetic defect which accelerated this process could be due to a loss of specificity of one of the many enzymes, or ribosomal proteins, which maintain the normal accuracy of protein synthesis. Most mutations of this type, however, would be dominant or partially so. Recessive mutations are frequently associated with the loss of an enzyme activity; therefore, if the recessive genetic defect causing Werner's syndrome is due to a slight change in the accuracy of protein synthesis, one possibility is that an enzyme which modifies bases in tRNA or rRNA is inactive or absent.

We thank Dr Alex Comfort for his encouragement and V. M. McGuire for cultures of normal skin fibroblasts.

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Life span of erythrocyte membrane protein

IN most types of cells the protein constituents of the cytoplasm are constantly being degraded and synthesised¹⁻⁶. When the proteins in these cells are labelled with a protein precursor, the label disappears from the protein with first order kinetics. The amount of any protein in the cell then is regulated by the rate of its synthesis and degradation, and recent work indicates the importance of this process as a regulatory mechanism in mammalian cells. In contrast, when haemoglobin, which comprises 90% of the protein of the mature mammalian erythrocyte, is labelled, it does not decay with first order kinetics, but survives for a finite period. It

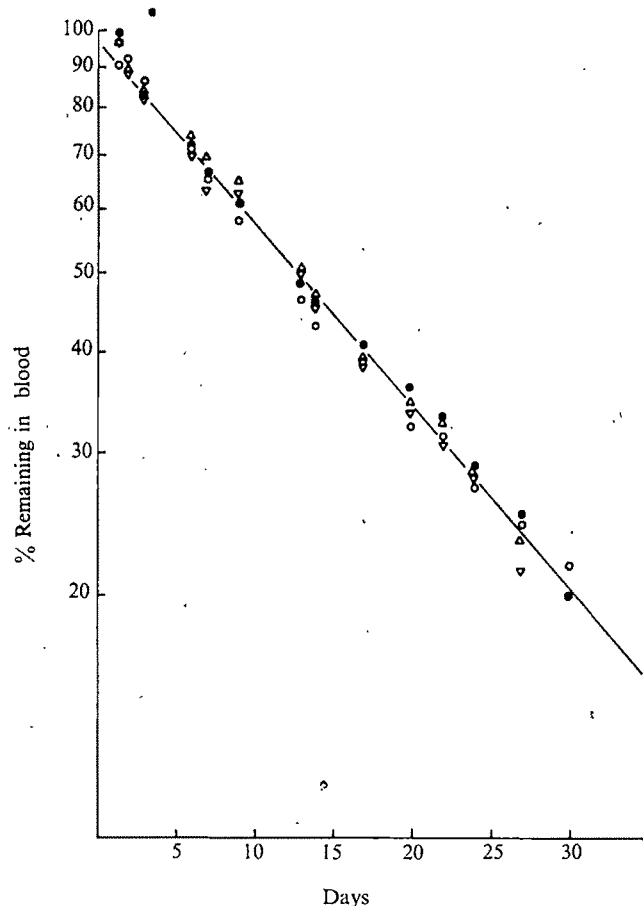


Fig. 1 Survival curves of ^{51}Cr and ^{125}I -labelled rat erythrocytes *in vivo*. Labelled cells (1 ml) were injected through the tail vein. At various times, blood samples were taken by tail cut and 0.05 ml of blood was collected into a heparinised capillary tube and mixed with 1.0 ml of normal saline. Samples were counted on a Packard gamma spectrometer. Four combinations of isotope were used: \circ , ^{125}I ; \bullet , ^{125}I Cr; Δ , ^{51}Cr I; and ∇ , ^{51}Cr . Each point is an average of data for four rats corrected for the decay of the isotope used.

disappears from the vascular bed when the intact erythrocyte is removed from circulation. Thus, haemoglobin labels provide excellent material for studies of the life span of the red cell.⁷

Membrane proteins also turn over in most animal cells. In the liver, for example, the half life of the plasma membrane proteins is very short¹⁻⁶. Efforts to label the membrane proteins of the erythrocyte and determine whether it survives similarly for the lifetime of the cell have not been completely successful⁸. Using ^{14}C -glucose which is incorporated into glycopospholipids, Krivit⁹, however, found a mean life span for rabbit red cells of 51 to 60d. These values are similar to but shorter than those obtained by labelling haemoglobin in the rabbit¹⁰. We have now labelled rat erythrocytes by a method previously described¹¹⁻¹³ and have studied the survival of their membrane proteins *in vivo*.

Male Sprague Dawley rats (200 \pm 10 g) were fed a regular diet, given water *ad lib* and housed four or five to a cage. Blood was drawn into plastic syringes containing ACD anticoagulant (0.15 ml ml⁻¹ of blood) from rats anaesthetised with ether. The blood was then placed in plastic tubes. The red cells were separated by centrifugation at 750g for 10 min and the serum was removed. For labelling with chromium^{14,15}, packed cells were obtained from 10 ml of blood. Potassium chromate (10A, 1 mg ml⁻¹) was added with stirring. After incubation at 37°C for 40 min, 0.1 ml of 0.1 M sodium ascorbate solution was added. The cells were incubated for a further 10 min and washed three times in isotonic saline.

To label cells with ^{125}I , packed cells were washed free of

plasma protein in isotonic phosphate buffer. The washed cells were then subjected to lactoperoxidase-catalysed iodination as previously described¹¹⁻¹³, centrifuged, washed three times in isotonic saline, suspended in serum and left overnight. Cells were then centrifuged free of the serum, washed twice in isotonic saline and suspended in saline to give a 50% haematocrit. The cells were also double labelled with either ^{125}I and non-radioactive chromium or ^{51}Cr and non-radioactive iodine. In all cases chromium labelling preceded labelling with iodide.

The cells from four groups of rats, consisting of four animals each were labelled with ^{125}I , ^{51}Cr , ^{125}I and non-radioactive chromium, or ^{51}Cr and non-radioactive iodine, as described above. A sample of 1 ml of cells was injected into the tail vein of each animal, and blood samples were obtained at various times thereafter. The amount of label present in the blood of each of the four groups was followed for 1 month.

As much as 25% of the total label rapidly disappeared within the first few hours after injection of cells. However, the rate of decay became more linear after 20h. Figure 1 shows the decay curve for the disappearance of radioactive label, corrected for the half-life of the isotopes used, from the four groups of animals used. The similarity in the rate of decay between ^{51}Cr and ^{125}I suggests that the label in exposed membrane proteins and that of internal components were disappearing in parallel.

Because ^{51}Cr elutes from the red cell it does not provide a simple relationship to the mean red cell life span^{14,15}. Survival times determined by chromium underestimate the mean red cell life span unless corrected. Since the disappearance of membrane label coincided with chromium decay, the data suggested that iodine was also being lost from the cell. To investigate whether any one of the membrane components is involved in a selective loss of an iodine-labelled component, erythrocyte membranes were isolated and polypeptides were separated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis.

Previous studies has shown that two proteins on the exterior surface of the human erythrocyte are labelled with the lactoperoxidase system¹¹⁻¹³. One has a molecular weight of 90,000 and the other is the major glycoprotein. In the rat, five polypeptides are iodinated. The major component labelled is in the 90,000 dalton class. The minor components have molecular weights of 68,000, 31,000, 18,000 and include an additional component with a low molecular weight, probably 12,000 or smaller.

As Fig. 2 shows, although there was a marked loss of radioactivity, there does not seem to have been a selective loss of any of the components. The minor components disappeared or were not readily detected after 404 h, because of the small amount of iodine originally incorporated.

The data suggest that labelling of the erythrocyte with the lactoperoxidase probe provides a method for the study of red cell survival, but does not offer any distinct advantages over present procedures. The labelling procedure is somewhat more complicated than the use of chromium. The iodine label gives results which indicate that a significant portion of the iodine label is removed from the cell during its life in the vascular system. The reasons for this are not clear. Chromium and other labelling systems label primarily the haemoglobin molecule which does not turn over as do proteins in other cell types, but survives for the life span of the red cell and is lost primarily by removal of the intact cell.

In other types of cells there is a marked difference in the rate of turn over of individual proteins of a membrane system¹⁻⁶. Analysis of the membrane components of the rat erythrocyte does not suggest any great difference in the rate of disappearance of the labelled membrane polypeptides. In other types of cell there seems to be a correlation between the size of a protein and its rate of degradation *in vivo*. The

(Continued on page 789)

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β -D-deoxy-ribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furbert's² model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furbert's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally^{3,4} that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{5,6} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

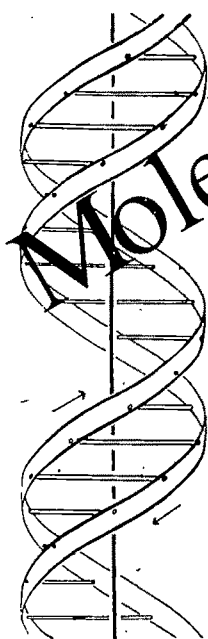
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April 2.

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This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis. There is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The double helix: a personal view

Francis Crick

Medical Research Council Laboratory for Molecular Biology, Hills Road, Cambridge, UK

Francis Crick reviews the papers published 21 years ago on the structure of DNA and the reaction to them.

For this anniversary I thought it might be appropriate to look back, in a rather informal way, at the original papers on the structure of DNA to see how they appear today in the light of 21 years of research.

During the spring and summer of 1953 Jim Watson and I wrote four papers on the structure and function of DNA. The first appeared in *Nature* on April 25 accompanied by two papers from King's College London, the first by Wilkins, Stokes and Wilson, the other by Franklin and Gosling. Five weeks later we published a second paper in *Nature*, this time on the genetic implications of the structure. A general discussion was included in the volume that came from that year's Cold Spring Harbor Symposium, the subject of which was viruses. We also published a detailed technical account of the structure, with rough coordinates, in an obscure journal¹ in the middle of 1954.

The first *Nature* paper was both brief and restrained. Apart from the structure itself the only feature of the paper which has excited comment was the short sentence: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material". This has been described as 'coy', a word that few would normally associate with either of the authors, at least in their scientific work. In fact it was a compromise, reflecting a difference of opinion. I was keen that the paper should discuss the genetic implications. Watson was against it. He suffered from periodic fears that the structure might be wrong and that he had made an ass of himself. I yielded to his point of view but insisted that something be put in the paper, otherwise someone else would certainly write to make the suggestion, assuming we had been too blind to see it. In short, it was a claim to priority.

Why, then, did we change our minds and, within only a few weeks, write the more speculative paper of May 30? The main reason was that when we sent the first draft of our initial paper to King's College we had not yet seen their own papers. Consequently we had little idea of how strongly their X-ray evidence supported our structure. The famous 'helical' X-ray picture of the B form, reproduced by Franklin and Gosling in their paper, had been shown to Watson, but he certainly had not remembered enough details to construct the arguments about Bessel functions and distances which the experimentalist gave. I myself, at that time, had not seen the picture at all. Consequently we were mildly surprised to discover that they had got so far and delighted to see how well their evidence supported our idea. Thus emboldened, Watson was easily persuaded that we should write a second paper.

The papers in *Nature*

The two experimental papers of April 25 overlap to a considerable extent. Rosalind Franklin's paper mentions the

crystalline A structure, but only briefly, except for the claim that the Patterson superposition function (which was in the press at the time) supported two chains rather than three. Both papers stress that there must be more than one chain in the structure. Indeed Maurice Wilkins had personally told Chargaff that a year or so earlier. Both present the argument that the positions of the intensity maxima ruled out two (parallel) chains related by a dyad parallel to the fibre axis. Neither gave the neat argument, due to Watson, that their own density measurement, together with the observed change in length between the two forms, supported two chains rather than three. Franklin noted that if there were several chains they could not be equally spaced and that 'equivalence' favoured two rather than three. It was not explicitly stated, however, that equivalence implies dyad axes perpendicular to the fibre axis and that therefore the two chains must run in opposite directions. Nor did she realise that the monoclinic unit cell of the A form also suggested this, although we had deduced this from her own experimental data.

Both papers correctly concluded from the intensity positions that the phosphate-sugar backbone was on the outside of the structure and that the bases were stacked on the inside. Franklin repeated the argument, which she had made to us verbally a year earlier, that the phosphates would be hydrated (in which she was perfectly right) and therefore that they would probably be on the outside of the molecule. In short, both the groups at King's College had obtained a fairly general idea of the structure but they had done no proper model building. Mainly because of this they had missed the pairing of the bases and they had completely overlooked the significance of Chargaff's rule.

The omissions in the paper by Watson and myself are also striking. The structure is produced like a rabbit out of a hat, with no indication as to how we arrived at it. No dimensions are given (let alone coordinates) except that the base pairs were 3.4 Å apart and that the structure had 10 base pairs in its repeat. The exact nature of the base pairing was not immediately obvious; nor even unambiguous since at that time there were two systems for numbering pyrimidine rings. Most of this information was provided in the subsequent papers. However the general nature of the structure was clear enough, though the tone of the paper ("it must be regarded as unproved until it has been checked against more exact results") was, apart from the short first paragraph, rather muted.

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Although a casual reader could easily have overlooked the significance of the first set of papers, especially as they were full of obscure crystallographic jargon, he could hardly miss the impact of our second one. The biologically important features of the proposed structure were explicitly described. The base pairs were listed with the minimum of hedging about tautomerism, and were illustrated in scale diagrams. The proposed duplication mechanism was spelt out in simple terms, unmarred by any trace of algebra. In spite of the discussion of the difficulties of unwinding, the list of unsolved problems and the reservations about the unproved nature of the structure, the final paragraph leaves little doubt that the authors thought they had a good idea.

How do they stand today?

How have these early papers stood the test of time? It can now be taken as firmly established that DNA usually consists of two chains, wound together and running in opposite directions. The evidence for this statement is so extensive that it would take too long to quote it all here. The fact that normally A pairs with T, and G with C, is also well established but the details were less certain until recently. The G:C pair was never in serious doubt. Watson and I drew this with only two hydrogen bonds but mentioned in our technical paper¹ that three was also a possibility. This was made almost certain by the theoretical arguments of Pauling and Corey² and was confirmed by X-ray structure determinations of single crystals of base pairs. The same technique showed that the A:T (or A:U) pair in single crystals usually did not have the configuration Watson and I suggested. The matter was only finally resolved about a year ago when Rich and his colleagues published two crystal structures; that of GpC paired with itself³ and ApU paired with itself⁴ (the backbone in each case was ribose), both to about 0.9 Å. They show not only the expected configurations for the base pairs but also make it highly likely that, as we claimed, nucleic acid helices are right handed.

In 1953 it was uncertain whether RNA could form a double helix. Watson and I stated that we thought we could not build our model for the B form of DNA with an RNA backbone. The discovery of double-stranded RNA viruses proved, however, that biological RNA too could form a double helix, though with slightly different parameters. The detailed coordinates we had (tentatively) suggested for DNA were soon shown to be incorrect (we had put the backbone at too big a radius) and much more accurate coordinates were provided by Wilkins and his colleagues, using fairly sophisticated methods of handling their much improved X-ray data. The general correctness of this work has been strongly supported recently by the single-crystal studies, mentioned above, of Rich and his coworkers.

Recently, Bram⁵ has put forward evidence that the parameters of a DNA double helix may vary somewhat with base composition, though whether this is a trivial variation or has deep biological implications is at present uncertain. Watson and I were so impressed with the apparent uniformity of the double helix from different biological sources and the regularity of the backbone of our model that we had no hesitation in saying that it "seems likely that the precise sequence of the bases is the code that carries the genetic information", an idea which gave me plenty to think about in the next 10 or 12 years.

Nothing was said about the possibility that the two chains might be melted apart and then annealed together again, correctly lined up. The discovery of this by Marmur and Doty has provided one of the essential tools of molecular biology. I can still remember the excitement I felt when Paul Doty told me about it at breakfast one day in New York in a hotel overlooking Central Park. But in other respects we were almost too far sighted, as witness our remark that recombination would probably depend upon

base pairing. We struggled for several years to produce neat models for this, all to no avail, partly because we accepted copy choice too easily but also because we were trying to invent a mechanism which did not need additional enzymes. This showed a gap in our overall grasp of molecular biology, which can also be glimpsed in our tentative suggestion that DNA synthesis might not need an enzyme, a remark I should certainly not make today except perhaps in the context of the origin of life.

As to DNA replication, our earliest description was mainly schematic. We realised that plain nucleotides were not likely to be the immediate precursor but missed the rather obvious idea that they were nucleoside triphosphates, again a lack of insight into biochemistry. We did suggest the so-called Y mechanism (in the Cold Spring Harbor paper) but did not mention the difficulties due to the direction of synthesis of antiparallel chains, though I frequently emphasised it a few years later. Looking back, I think we deserve some credit for not being inhibited by the difficulty of unwinding which we clearly recognised and for our forthright stand against paranemic (as opposed to plectonemic) coiling. In this instance our grasp of X-ray diffraction was invaluable.

The functions of DNA

It is, of course, somewhat a matter for surprise that DNA synthesis is not fully understood even today. It would take too much space to discuss the complex and rapidly moving field here. Semiconservative replication in many instances is firmly established. The process certainly occurs as if base pairing were taking place, but I have often asked myself what evidence would make it certain that base pairing really occurs rather than some elaborate allosteric mechanism, even though the latter seems unlikely. Perhaps only an X-ray determination of the structure of the polymerase will finally answer the question. Meanwhile the topics of Okazaki fragments, rolling circle models, RNA primers and the exact roles of the various polymerases will keep many people busy. Even at that early period we did at least ask whether the DNA of a chromosome was in one long molecule, though the idea of circular DNA never occurred to us. Nor did we suggest that a virus might have single-stranded DNA. There is however one remark which may turn out to be perspicacious "... we suspect that the most reasonable way to avoid tangling is to have the DNA fold up into a compact bundle as it is formed". As we struggle with the structure of the *E. coli* chromosome and the even more formidable problem of the structure of the chromosomes of higher organisms—probably the major unsolved problem of molecular biology today—it might be worth remembering this tentative suggestion from the distant past.

The other topic we touched on was mutation. This was of the base-substitution type—there is no hint of frameshift mutants. We totally missed the possible role of enzymes in repair although, due to Claud Rupert's early very elegant work on photoreactivation, I later came to realise that DNA is so precious that probably many distinct repair mechanisms would exist. Nowadays one could hardly discuss mutation without considering repair at the same time.

There is no hint in these early papers that nucleic acid might form a complex three-dimensional structure such as we now find in transfer RNA nor even the idea of the hypothetical Gierer loops. Our message was that DNA was simple and alone carried the genetic information. We saw no reason to complicate it till we had to. For the same reason although we must have drawn a G:U pair we attached no importance to it. "Wobble" was still far in the future, but these, it seems to me, are forgivable oversights.

Reactions to the structure

It is really for the historian of science to decide how our structure was received. This is not an easy question to

answer because there was naturally a spectrum of opinion which changed with time. There is no doubt, however, that it had a considerable and immediate impact on an influential group of active scientists. Mainly due to Max Delbrück, copies of the initial three papers were distributed to all those attending the 1953 Cold Spring Harbor Symposium and Watson's talk was added to the programme. A little later I gave a lecture at the Rockefeller which I am told produced considerable interest, partly I think because I mixed an enthusiastic presentation of our ideas with a fairly cool assessment of the experimental evidence, roughly on the lines of the article which appeared in *Scientific American* in October, 1954. Sydney Brenner, who had just finished his PhD, at Oxford under Hinshelwood, appointed himself, in the summer of 1954, as Our Representative at Cold Spring Harbor and took some pains to get the ideas over to Demerec. It was about this time that Matt Meselson, just moving into biology from physical chemistry, grasped the importance of inventing a new method to tackle the problem of semiconservative replication, a theoretical analysis which led to density gradient centrifugation. But not everyone was convinced. Barry Commoner insisted, with some force, that physicists oversimplified biology, in which he was not completely wrong. Chargaff, when I visited him in the winter of 1953-54, told me (with his customary insight) that while our first paper in *Nature* was interesting, our second paper on the genetic implications was no good at all. I was mildly surprised to find, when, some years later, in 1959, I talked with Fritz Lipmann who had arranged that I should give a series of lectures at the Rockefeller, that he had not really grasped our scheme of DNA replication. (It emerged that he had been talking to Chargaff.) By the end of the lectures, however, when he summed up, he gave a remarkably clear outline of our ideas. Arthur Kornberg has told me that when he began work on DNA replication he did not believe in our mechanism, but his own brilliant experiments soon made him a convert, though always a careful and critical one. It was his work which produced the first good evidence that the two chains run in opposite directions. All in all it seems to me that we got a very fair hearing, better than Avery and certainly a lot better than Mendel.

Not that it was all plain sailing. We were naturally delighted with the work of Meselson and Stahl, and of Herbert Taylor, on semiconservative replication, though I have never thought this the essence of our ideas which lies rather in the base pairing. Seymour Benzer's genetic analysis of the r_{II} locus of phage T4 encouraged us greatly. But we had to live through the claims of Marshak that there was no DNA in *Arbacia* eggs and of a Canadian group that the amount of DNA synthesis in one cell cycle was twice the expected amount. At a later stage Cavalieri claimed that the basic DNA structure had four chains, rather than two, an idea which cropped up again more recently. On the crystallographic side Donohue, whose advice had been crucial to our understanding of base pairing, was a persistent critic of the validity of the later X-ray work, but in recent years he carried it too far, refusing, for example, to admit as evidence the great accumulation of data showing that the two chains are antiparallel. (In 1956, he had rashly published, with Stent, a quite erroneous structure having like-with-like pairing.) I hope the recent papers by Rich, referred to above, have to some extent reduced his doubts, which at times had some justification.

Who might have discovered it?

Then there is the question, what would have happened if Watson and I had not put forward the DNA structure? This is 'iffy' history which I am told is not in good repute with historians, though if a historian cannot give plausible answers to such questions I do not see what historical analysis is

about. If Watson had been killed by a tennis ball I am reasonably sure I would not have solved the structure alone, but who would? Olby⁶ has recently addressed himself to this question. Watson and I always thought that Linus Pauling would be bound to have another shot at the structure once he had seen the King's College X-ray data, but he has recently stated that even though he immediately liked our structure it took him a little time to decide finally that his own was wrong. Without our model he might never have done so. Rosalind Franklin was only two steps away from the solution. She needed to realise that the two chains must run in opposite directions and that the bases, in their correct tautomeric forms, were paired together. She was, however, on the point of leaving King's College and DNA, to work instead on TMV with Bernal. Maurice Wilkins had announced to us, just before he knew of our structure, that he was going to work full time on the problem. Our persistent propaganda for model building had also had its effect (we had previously lent them our jigs to build models but they had not used them) and he was proposing to give it a try. I doubt myself whether the discovery of the structure could have been delayed for more than two or three years.

There is a more general argument, however, recently proposed by Gunther Stent and supported by such a sophisticated thinker as Medawar. This is that if Watson and I had not discovered the structure, instead of being revealed with a flourish it would have trickled out and that its impact would have been far less. For this sort of reason Stent had argued that a scientific discovery is more akin to a work of art than is generally admitted. Style, he argues, is as important as content.

I am not completely convinced by this argument, at least in this case. Rather than believe that Watson and Crick made the DNA structure, I would rather stress that the structure made Watson and Crick. After all, I was almost totally unknown at the time and Watson was regarded, in most circles, as too bright to be really sound. But what I think is overlooked in such arguments is the intrinsic beauty of the DNA double helix. It is the molecule which has style, quite as much as the scientists. The genetic code was not revealed all in one go but it did not lack for impact once it had been pieced together. I doubt if it made all that difference that it was Columbus who discovered America. What mattered much more was that people and money were available to exploit the discovery when it was made. It is this aspect of the history of the DNA structure which I think demands attention, rather than the personal elements in the act of discovery, however interesting they may be as an object lesson (good or bad) to other workers.

My own reactions

I have sometimes been asked whether I had ever contemplated writing my own account of the discovery. In the 1950s I did give a lecture on this subject to a group of historians of science at Cambridge and to a similar group at Oxford. I was able to be rather more scholarly than Watson could allow himself in *The Double Helix*, which is better regarded as a rather vivid fragment of his autobiography, written for a lay audience. As to a book I confess I did get as far as composing a title (*The Loose Screw*) and what I hoped was a catchy opening ("Jim was always clumsy with his hands. One had only to see him peel an orange. . .") but I found I had no stomach to go on. Recently we made a film together about it for undergraduates. Much had to be left out when the film came to be cut but it does to some extent supplement Jim's book. Since Olby's detailed and scholarly account⁶ will soon be available I doubt if there is now much more I can usefully add.

Finally one should perhaps ask the personal question—am I glad that it happened as it did? I can only answer that

I enjoyed every moment of it, the downs as well as the ups. It certainly helped me in my subsequent propaganda for the genetic code. But to convey my own feelings, I cannot do better than quote from a brilliant and perceptive lecture I heard years ago in Cambridge by the painter John Minton (he later committed suicide) in which he said of his own artistic creations "the important thing is to be there when the picture is painted". And this, it seems to me, is partly a matter of luck and partly good judgement, inspiration and persistent application.

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Molecular basis of biological specificity

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Linus Pauling reviews his work on the molecular basis of biological specificity and remembers his erroneous conception of a three-chain helix structure for DNA in 1952.

DURING the decade 1930-40 I formulated a general theory of the molecular basis of biological specificity, involving the idea that biological specificity results from the interaction of complementary molecular structures, with hydrogen bonds among the most important of the weak intermolecular forces between the interacting molecules. The most striking example of specific biological interactions of this sort is the interaction between the two complementary strands of the DNA molecule in the double helix discovered by Watson and Crick 21 years ago.

Early work

My early work was on the determination of the structure of crystals by the X-ray diffraction technique, the determination of the structure of gas molecules by electron diffraction, and the application of quantum mechanics to physical and chemical problems, especially the structure of molecules and the nature of the chemical bond. In 1929, when Thomas Hunt Morgan came to the California Institute of Technology, bringing with him a number of very able younger biologists, I began to become familiar with biological problems, and to think about possible ways in which biological specificity could be explained in terms of interactions between molecules. I worked on several problems of biological specificity, from the molecular point of view, without success; one of them was the problem of explaining the self sterility of the marine organism *Ciona* (the sea squirt), which was being studied by Morgan. In 1934 the problem of the shape of the oxygen equilibrium curve of haemoglobin attracted my attention. Consideration of the structure of haemoglobin led to the idea that investigation of the magnetic properties of this substance and its derivatives would provide valuable information, and work along these lines, in collaboration with C. D. Coryell and a number of students, was initiated. Alfred E. Mirsky of the Rockefeller Institute for Medical Research, who had been studying haemoglobin for several years, came to Pasadena for a year, and he and I formulated a theory of the structure of native, denatured, and coagulated proteins, based upon the concept that a native protein molecule consists of one polypeptide chain (or of two or more such chains) folded into a uniquely defined configuration, in which

it is held by hydrogen bonds between the peptide nitrogen and oxygen atoms, as well as by other weak forces, with denaturation involving a loss of this well-defined structure¹.

Antigens and antibodies

In 1936, while I was on a short visit to the Rockefeller Institute for Medical Research, Karl Landsteiner asked me how I would explain the observed properties of antibodies and antigens by means of their molecular structure. I thought about this problem during the following years, and consulted Landsteiner about the interpretation of sometimes conflicting experimental results. By 1940 I had formulated a theory of the structure, and process of formation of antibodies². This theory was based upon the concept that the specific combining region of an antibody molecule is complementary in structure to a portion of the surface of the antigen, with the antigen-antibody bond resulting from the cooperation of weak forces (electronic Van der Waals forces, electrostatic interaction of charged groups, and hydrogen bonding) between the complementary structures, over an area sufficiently large that the total binding energy could resist the disrupting influence of thermal agitation. Precipitating and agglutinating antibodies were assumed to be bivalent, consisting of a central part, with structure common to all or almost all antibodies produced by the animal, and two end parts, the combining regions, with structure complementary to that of the antigen. (The idea of complementary structures for antibody and antigen was suggested by Breml and Haurowitz³, Alexander⁴, and Mudd⁵. There is some intimation of it in the early work of Ehrlich and Bordet.) The complementary combining regions were assumed to be formed by the folding of polypeptide chains in the presence of the antigen, in such a way that the forces of attraction would mould the folding chain into a structure complementary to that of a portion of the antigen, with the folded chain then being held in this configuration by hydrogen bonds and other interactions, even after the antibody has dissociated from the antigen on which the combining group was moulded. Dan Campbell, David Pressman, and a number of other workers in our laboratory carried out experimental studies that verified the valence 2 for precipitating and agglutinating antibodies^{6,7} and that left no doubt that the combining regions of antibodies are complementary in structure to the homologous haptens or groups of the antigen⁸. The fit of the combining region of the antigen to the hapten was shown to be close, better than 20 pm in some cases, and the effects of Van der Waals attraction, electrostatic forces, and hydrogen-bond formation were

separately verified in quantitative hapten-inhibition studies. A satisfactory theoretical explanation of quantitative values of free energy of combination of haptens with antibodies homologous to the *o*-, *m*-, and *p*-azobenzene arsenic acid groups on the basis of known intermolecular interactions was reported in 1945 (ref. 9). For several haptens with various groups substituted in the positions of the azo group in the hapten of the immunising antigen the standard free energy of combination, as given by hapten inhibition constants, was found to be proportional to the calculated Van der Waals interaction with the surrounding antibody, which includes proportionality to the electric polarisability of the group. For groups forming hydrogen bonds the energy of the hydrogen bond (1.5 to 3 kJ mol⁻¹, representing the difference in energy of the hydrogen bond formed by the hapten with antibody and with water) was needed, in addition to the term corresponding to electronic Van der Waals interaction. The effect of electric charge was determined by comparison of haptens closely similar in shape, but with a difference in electric charge: in one case¹⁰ comparison of haptens with either trimethylammonium ion or tertiary butyl group, and in the other case¹¹ with either carboxylate ion or nitro group. In each comparison there was indication of a complementary electric charge in the antibody, close to the charge in the immunising antigen. The magnitude of the effect showed the charge in the antibody to be within 320 pm (first case) or 260 pm (second case) of the minimum distance permitted by the Van der Waals radii of the groups. I think that this work, which was based on earlier work by Landsteiner and his collaborators¹², leaves no doubt that the specificity of antibodies is the result of the complementarity in structure of the combining group and a portion of the surface of the homologous antigen.

Nonbiological specificity

It became evident that nonbiological specificity could also be explained in terms of complementarity. I gave an example in a lecture on analogies between antibodies and simpler chemical substances¹³. "The reaction shown by simple chemical substances that is analogous to that of specific combination of antigen and antibody is the formation of a crystal of a substance from solution. A crystal of a molecular substance is stable because all of the molecules pile themselves into such a configuration that each molecule is surrounded as closely as possible by other molecules—that is, if a molecule were to be removed from the interior of a crystal, the cavity that it would leave would have very nearly the shape of the molecule itself. We can say that the part of a crystal other than a given molecule is very closely complementary to that molecule. Other molecules, with different shape and structure, would not fit into this cavity nearly so well, and in consequence other molecules in general would not be incorporated in a growing crystal. This is the explanation of the astounding chemical process of purification by crystallization—from a very complicated system, such as, for example, grape jelly, containing hundreds of different kinds of molecules, crystals which are nearly chemically pure may be formed, such as crystals of cream of tartar, potassium hydrogen tartrate".

In the same paper it is stated that "although crystallisation is the only simple chemical reaction that shows striking similarity to serological reactions with respect to specificity, there are many physiological phenomena that are similarly specific, and for which the specificity can be given a similar explanation. The specificity of the catalytic activity of enzymes is due to a surface configuration of the enzyme such as to make the enzyme complementary to the substrate molecule or, rather, to the substrate molecule in the strained state that occurs during the catalysed reaction. The specific action of drugs and bactericidal substances have a similar explanation. Even the senses of taste and odour are based

upon molecular configuration rather than upon ordinary chemical properties—a molecule which has the same shape as a camphor molecule will smell like camphor even though it may be quite unrelated to camphor chemically. I am convinced that it will be found in the future, as our understanding of physiological phenomena becomes deeper, that the shapes and sizes of molecules are of just as great significance in determining their physiological behavior as are their internal structure and ordinary chemical properties."

Intermolecular forces in biological processes

In 1940 Max Delbrück and I¹⁴ published a discussion of the intermolecular forces operative in biological processes. P. Jordan had advanced the idea that there exists a quantum-mechanical stabilising interaction that operates preferentially between identical or nearly identical molecules or parts of molecules, and is of great importance for biological processes, including the production of new genes identical with the old ones. Delbrück and I pointed out that the specific quantum-mechanical forces between identical molecules could not be large enough to cause a specific attraction between like molecules under the conditions of excitation and perturbation prevailing in living organisms, and therefore could not be effective in bringing about autocatalytic reactions. We wrote that "It is our opinion that the processes of synthesis and folding of highly complex molecules in the living cell involve, in addition to covalent-bond formation, only the intermolecular interactions of Van der Waals attraction and repulsion, electrostatic interactions, hydrogen-bond formation, etc., which are now rather well understood. These interactions are such as to give stability to a system of two molecules with complementary structures in juxtaposition, rather than of two molecules with necessarily identical structures; we accordingly feel that complementarity should be given primary consideration in the discussion of specific attraction between molecules and the enzymatic synthesis of molecules." We mentioned that "The case might occur in which the two complementary structures happened to be identical; however, in this case also the stability of the complex of two molecules would be due to their complementarity rather than their identity." Some time later¹⁵ I discussed the matter of gene replication in more detail: "I believe that the genes serve as the templates on which are molded the enzymes that are responsible for the chemical characters of the organisms, and that they also serve as templates for the production of replicas of themselves. The detailed mechanism by means of which a gene or a virus molecule produces replicas of itself is not yet known. In general the use of a gene or virus as a template would lead to the formation of a molecule not with identical structure but with complementary structure. If might happen, of course, that a molecule could be at the same time identical with and complementary* to the template on which it is molded. However, this case seems to me to be too unlikely to be valid in general, except in the following way. If the structure that serves as a template (the gene or virus molecule) consists of, say, two parts, which are themselves complementary in structure, then each of these parts can serve as the mold for the production of a replica of the other part, and the complex of two complementary parts thus can serve as the mold for the production of duplicates of itself." The same statements were made in the spring of 1948 in lectures in Oxford, Cambridge, London and elsewhere.

The hydrogen bond was recognised by Latimer and Rodebush as an important structural feature more than 50 years ago¹⁶. In their 1920 paper they mentioned that "Mr Huggins of this laboratory in some work as yet unpublished has used the idea of a hydrogen kernel held between two atoms as a theory in regard to certain organic compounds." In 1936 Mirsky and I pointed out the importance of the hydrogen bond in determining the structure of proteins¹. In the same

year Huggins also discussed protein structures in a more detailed way, with hydrogen bonds between the NH and CO groups of the main chains¹⁷. A few years later Huggins described several helical structures for polypeptide chains, with intrachain hydrogen bonds¹⁸. These structures were needlessly restricted to having an integral number of amino acid residues per turn of the chain and, moreover, Huggins did not require the amide groups to be planar, although the planarity of these groups had been recognised since 1932 (ref. 19), and had already been verified by several determinations of the structure of simple peptide crystals in our laboratory. It is unfortunate that Huggins was handicapped by these two erroneous assumptions in his imaginative and otherwise sound attack on the problem of the secondary structure of proteins. The same two erroneous assumptions provided a similar insuperable barrier to the vigorous attack made by Bragg, Kendrew, and Perutz on the same problem²⁰. In the meantime, Corey and other investigators in Pasadena had determined the crystal structures of a number of amino acids and simple peptides, and Corey and I had discovered the alpha helix and the parallel chain and antiparallel chain pleated sheets²¹. The discovery of the alpha helix left no doubt about the importance of helical structures and of hydrogen bonds in determining the secondary structures of proteins.

Nucleic acids

I had been interested in the nucleic acids since 1933, when Sherman and I calculated the resonance energy of guanine and other purines²². My colleague Robert B. Corey had made some X-ray diffraction photographs of fibres of nucleic acid, which were, however, of somewhat poorer quality than those published by Astbury and Bell²³. I began work on the problem of interpreting the X-ray photographs on November 26, 1952; on the preceding day I had attended a seminar in biology in the California Institute of Technology, at which Professor Robley Williams of University of California, Berkeley, showed a slide of an electron microscope photograph of molecules of sodium ribonucleate. He said that the small fibrils had a diameter of about 1.5 nm, and that they were apparently cylindrical, in that only one diameter was shown. The X-ray photographs indicated an identity distance along the axis of the molecule of 340 pm, and, with the measured density of RNA, about 1.62 g cm⁻³, it was indicated that the fibres contain two or three molecules, probably helices twisted about one another. The value of the spacing of the principal equatorial X-ray reflection had been shown to decrease with decreasing amount of hydration of the fibres, with a minimum value of 1.62 nm. I assumed this value to correspond to essentially anhydrous nucleic acid, and, using the density, I calculated the number of polynucleotide chains per unit to be exactly three. This result surprised me, because I had expected the value 2 if the nucleic acid fibres really represented genes. I decided, however, that probably the fibres were artefacts, produced by the process of extraction from cells and the subsequent stretching. During the next month I strove to find a way of arranging the polynucleotide chains in a triple helix, and was successful, although the structure was described as "an extraordinarily tight one, with little opportunity for change in positions of the atoms". The paper in which this structure was described was communicated to the *Proceedings of the National Academy of Sciences* on December 31, 1952, and a copy of the manuscript was sent to Watson and Crick²⁴.

In hindsight, it is evident that I made a mistake on November 26, 1952 in having decided to study the triple helix rather than the double helix. It is likely that the fibres giving the equatorial spacing 1.62 nm contained some water, and also had a density less than 1.62 g cm⁻³. The diameter 1.5 nm observed by Williams for nucleic acid molecules corresponds, with an assumed density of 1.6 g cm⁻³ and unit translation 340 pm along the molecular axis, to two molecules in a

helical structure (calculated diameter 1.6 nm) rather than to three (1.9 nm). I am now astonished that I began work on the triple helix structure, rather than on the double helix. I had not forgotten that Delbrück and I had suggested that the gene might consist of two complementary molecules, but for some reason, not clear to me now, the triple chain structure apparently appealed to me, possibly because the assumption of a three-fold axis simplified the search for an acceptable structure.

I cannot say what would have happened if I had made the other assumption, that of a double helix, on November 26, or if I had succeeded in getting access to the diffraction photographs of DNA that had been made by Wilkins. There is a chance that I would have thought of the Watson-Crick structure during the next few weeks. I knew that the purines and pyrimidines were present in nucleic acid in equal amounts, but I had not drawn the reasonable conclusion about purine-pyrimidine pairs. I knew about hydrogen bonding by purines and pyrimidines. Nevertheless, I myself think that the chance is rather small that I would have thought of the double helix in 1952, before Watson and Crick made their great discovery. After all, I had spent part of the summer of 1937 in a search for ways of folding polypeptide chains, with planar amide groups of the correct dimensions and with hydrogen bonds between the CO and NH groups of residues separated by some distance along the chain, in such a way as to account for the X-ray diffraction photographs of alpha keratin, but without success. There was no reason why the alpha helix should not have been discovered then, rather than 11 years later, when it was discovered after a few hours of work. There is no doubt that even rather simple ideas sometimes are very elusive.

It is my opinion that if Watson and Crick had not carried on their persistent effort, and had not had the benefit of advice about the structures of the nitrogen bases and hydrogen bonds from Jerry Donohue and information from the excellent X-ray diffraction photographs of Wilkins, the discovery of the double helix, which has led to such great developments in molecular biology, might well have been delayed for several years.

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Molecular biology in a living cell

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Dr Gurdon discusses the introduction of purified macromolecules, such as nucleic acids and proteins, into living cells in controlled amounts and in such a way that they function normally.

MUCH of the success of molecular biology during the past 20 years has stemmed from the development of methods for purifying macromolecules. Initially this opened the way towards a detailed description of the composition and three-dimensional structure of macromolecules. Subsequently, it enabled the function of identified cell components such as nucleic acid polymerases, messenger RNA and initiation factors, to be determined using cell-free systems which synthesise DNA, RNA or protein. There are however certain questions about the function and behaviour of macromolecules which are unlikely to be answered by a knowledge of their structure and of their behaviour in cell-free systems. These are questions, moreover, which could be answered if it were possible to introduce purified macromolecules into living cells in significant amounts and in such a way that they function normally. This article summarises what is known of the fate of purified macromolecules inserted into living cells, and outlines some of the present and future uses for this particular type of molecular biology.

Macromolecules do not enter cells at all readily under normal conditions¹ (see Fig. 1a). There are exceptions to this rule, such as the passage of some hormones into responsive cells, the uptake of RNA by macrophages and the events involved in virus infection, but in these cases cells possess special receptors or mechanisms for the selective uptake of a restricted range of molecules. Even when agents are used which increase incorporation, such as DEAE dextran^{2,3}, purified molecules, such as RNAs, are susceptible to breakdown if added to undefined media of the kind preferred by growing cells. Only a small proportion of macromolecules in the medium enter a cell over many hours and those which do may be partly degraded. A different approach to the problem is to inject a solution of macromolecules directly into a living cell. By this means, a known number of purified macromolecules can be inserted directly into a living cell without significant contact with the external medium. The manipulations required⁴ are relatively undemanding so long as large cells are used as recipients. Here special emphasis is given to injection experiments with amphibian eggs and oocytes; these cells are especially suitable for such experiments on account of their large size, which permits quantitative work, and their developmental potentiality, which, in the case of eggs, ensures that injected molecules are distributed to different kinds of specialised cells.

Experiments involving the injection of macromolecules into living cells fall into two categories. The first includes attempts to validate injection experiments by proving that introduced macromolecules are accepted in a normal and functional way by the recipient cells. The second involves the application of this technique to specific questions about the characteristics of the injected molecules and the recipient cells.

Validity of injection experiments

We need to know that injected molecules are not rapidly

broken down or eliminated from cells, that they are not toxic in moderate amounts, that they are distributed in cells in their characteristic way, and that they function normally. The most exacting test of these requirements is provided by following the fate of injected macromolecules which have been purified away from other molecules with which they are always associated in cells. As far as is known such molecules as messenger RNA and DNA never exist in cells free and uncomplexed with proteins. Another kind of sensitive test

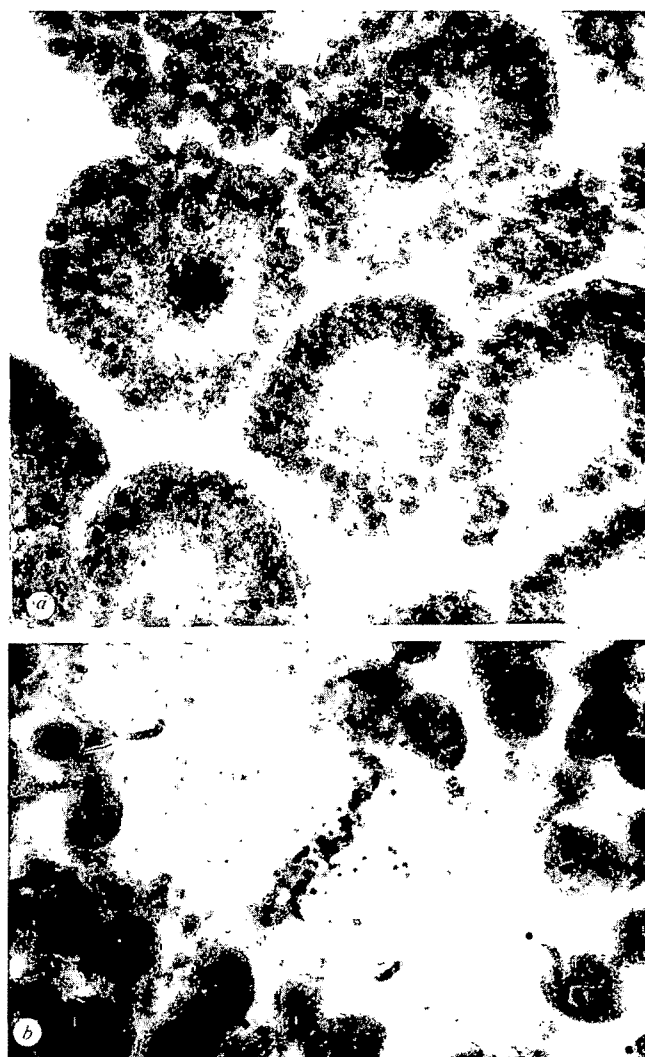


Fig. 1. Autoradiographs of sections of blastulae grown from eggs in which one of the blastomeres was injected at the four-cell stage with *Xenopus* erythrocyte histones labelled with ¹²⁵I. a, Two cleavage cells, which are presumed to be daughters of the same parent cell, and which have intensely labelled nuclei. The neighbouring cells, presumed to have been derived from uninjected blastomeres, are completely unlabelled. Evidently labelled histones do not pass freely across cell walls. b, Autoradiographic grains over chromosomes on a metaphase spindle. Some of the injected ¹²⁵I-histones appear to have become associated with chromosomes. These are the results of unpublished experiments with W. M. Bonner.

TABLE 1 Tolerance of fertilised *Xenopus* eggs to injected macromolecules

Injected molecules			Survival§, ¶		Relative increase§,		
Type	No. per egg	Weight per egg†	% injected Blastula (st. 9)	eggs surviving to Tadpole (st. 40)	Injected molecules as a proportion of embryos' normal content of equivalent molecules		
					Fertilised egg (st. 1)	Blastula (st. 9)	Tadpole (st. 40)
Proteins*							
Total histones (<i>Xenopus</i>)	5×10^{12} 5×10^{11}	100 ng 10 ng	Mostly abnormal 85%	4% 52%	0.2	0.05	0.005
					(relative to total histone)		
Histone f2a2 (calf thymus)	5×10^{11}	10 ng	94%	67%	1.0	0.25	0.025
					(relative to histone f2a2)		
Myoglobin (whale)	5×10^{11}	20 ng	83%	72%	Myoglobin probably absent from eggs and blastulae		Probably low in tadpoles
RNA†							
Globin mRNA (rabbit or mouse)	5×10^{10}	18 ng	82%	41%	1.0	1.0	0.2
					(relative to total poly (A)-mRNA)		
DNA							
Total chromosomal	2×10^8	4 ng	Mostly abnormal	2%	100	0.005	0.0004
(native calf thymus)	5×10^7	1 ng		84%			
(10 ⁷ daltons)							
Polyoma virus (form I native closed circles)	5×10^6	0.02 ng	88%	40%			
Injection medium							
Control	—	—	90%	86%	—	—	—

This table shows the upper limits of tolerance of fertilised eggs, which are more sensitive than oocytes, to introduced macromolecules. Survival to the tadpole stage is a sensitive test of toxicity (see below). Some frog species have larger eggs with greater tolerances.

* Unpublished experiments of W. M. Bonner and J. B. G.

† Refs 8 and 22.

‡ Each sample was injected in a volume of 20 nl per egg.

§ For stage numbers see ref. 43.

¶ Up to 5% survival to tadpoles may be attributed to eggs in which excessive leakage has occurred, and which are not therefore a proper test of toxicity. Survivals of 75% or more are not distinguishable from total nontoxicity, because some eggs are congenitally abnormal. || Values for the normal content of proteins and mRNA are approximate. The histone content of cleavage embryos is certainly in considerable excess of their nuclear DNA content but may be smaller than the values used to make these calculations (see ref. 27). Eggs contain ~30 ng of poly(A)-containing RNA⁴⁴, but only ~2% of this is being translated on polysomes (H. R. Woodland, unpublished). Relative to fertilised eggs blastulae contain twice as much, and tadpoles about 20 times as much, polysome-associated mRNA (H. R. Woodland, unpublished). Leakage and breakdown (see text) have not been allowed for in comparing the amounts of injected and endogenous molecules.

is provided by following the fate of injected molecules which normally have a characteristic intracellular distribution; examples of these are histones which are synthesised in cell cytoplasm but which very rapidly enter and accumulate in the nucleus⁵.

The leakage or rapid breakdown of injected molecules has been tested by following the fate of ³H-labelled DNA or ¹²⁵I-labelled histones. The disappearance of acid-insoluble label often amounts to 20–30% within the first few hours after injection, but after this its rate of loss is less than 10% per day. Experiments with globin mRNA (below) suggest that it has a half life of more than one week. The electrophoretic analysis of material extracted from tadpoles 3 days after they were injected, at the egg stage, with ¹²⁵I-histone fractions F2a1 and F2b showed the presence of these two proteins as the major labelled components (W. M. Bonner, unpublished experiments involving the injection of separated fractions of calf thymus histones and unfractionated *Xenopus* erythrocyte histones into oocytes and eggs). Apparently most of the injected proteins and nucleic acids are not rapidly eliminated or broken down.

Toxicity tests have been carried out to determine the numbers of different molecules that can be tolerated by oocytes and eggs. If fertilised eggs, which are much more sensitive than oocytes, can develop into normal tadpoles, the injected molecules are considered nontoxic. Table 1 shows the maximum amounts tolerated by a *Xenopus* egg, that is about 1 ng of DNA, and 10–20 ng (~10¹¹ molecules) of proteins or RNA. These numbers of molecules are entirely sufficient for most experimental analyses to be carried out on a small number of eggs (about 10).

The tendency of injected molecules to take up their normal intracellular distribution has been examined by following autoradiographically the fate of ¹²⁵I-histones injected into oocytes and eggs. Labelled histones show a spectacular accumulation in the nucleus of an oocyte; after 12 h they are

about 100 times more concentrated in the nucleus than in the cytoplasm⁶, a situation which can result in the nucleus containing several hundred times more than its normal content of histone associated with DNA. Histones also accumulate strongly in the nuclei of fertilised cleaving eggs (Fig. 1a). The same nuclear accumulation is not shown by nonnuclear proteins such as bovine serum albumin, nor by proteins of the same size range (myoglobin) or charge (lysozyme) as histones (W. M. Bonner, unpublished). The examination of metaphase chromosomes suggests that at least some of the injected histones become associated with chromosomes (Fig. 1b). Separated calf thymus histone fractions give broadly similar results to those just outlined for *Xenopus* erythrocyte histones. Some of the injected mRNA seems also to take up its normal location in injected cells, since globin mRNA becomes incorporated into polysomes (mostly pentasomes)⁷ and its translation is inhibited by cycloheximide⁸. The fate of DNA injected into fertilised eggs is now under investigation, but other work on DNA which enters cultured plant or animal cells has shown that some of the DNA is included in the nucleus^{9,10}, and may be linked to chromosomal DNA^{11,12}.

Evidence for the normal function of injected macromolecules has come from experiments with RNA and DNA, since the function of histones is not known. Results obtained with globin mRNA have shown conclusively that some of the injected molecules can function normally. α and β globin mRNAs of the rabbit, mouse and duck are all translated accurately, as judged by several different types of analysis of oocyte-synthesised haemoglobin, globin and globin peptides^{13–15}. Furthermore, the injected mRNA is used efficiently; appropriate calculations¹⁶ suggest that, on average, one globin molecule is synthesised in oocytes from each mRNA every 2 min. This is about four times less often than would have been the case in rabbit reticulocytes at 19° C. If, however, some of the injected molecules leak out, are broken down, or

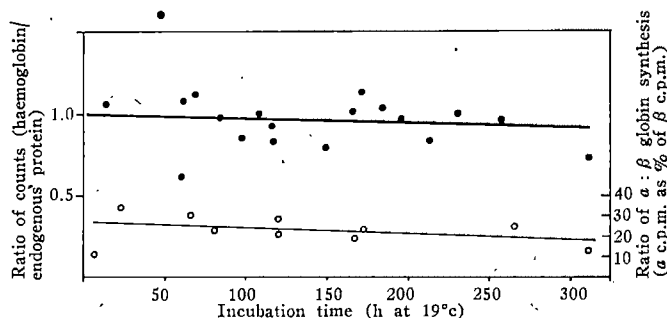


Fig. 2 Stability of globin messenger RNA in *Xenopus* oocytes. Purified mouse globin mRNA (7 ng per oocyte) was injected into several groups of oocytes which were incubated in unlabelled medium. At intervals, a group was transferred to ^3H -histidine-containing medium for 12 h, and then analysed for the ratio of labelled haemoglobin to labelled endogenous proteins (●) and for the ratio of α globin to β globin synthesis (○). Details in ref. 16.

are defective, the efficiency with which some of the injected molecules are translated would be close to what is normal for the cells from which they were prepared.

Evidence for the function of injected DNA has been obtained for replication, though not as yet for transcription. The strongest case for replication of DNA has come from the injection of double-stranded supercoiled polyoma virus DNA into eggs¹⁷. The tests carried out include the demonstration that heavy:light parental molecules, density-labelled with BUDR on one strand, cause the appearance in eggs of light:light double-stranded molecules. ^3H -TdR incorporation was observed into new strands which were complementary, and hydrogen bonded, to the density-labelled parental strands. Since the newly synthesised polyoma DNA was selectively extracted, and purified in alkaline sucrose gradients as 53S molecules, it is evidently present as closed supercoiled circles. The egg cytoplasm has apparently been able to open the parental supercoils, synthesise a copy semiconservatively, separate the newly synthesised molecules, and close them up into circles. It seems that different kinds of vertebrate native DNA will serve as a template for replication in egg cytoplasm¹⁸, but do so to a much lesser extent in oocyte cytoplasm^{19,20}. This difference is important since the behaviour of injected molecules reflects the normal metabolic difference between oocytes and eggs in respect of DNA synthesis, a result which gives confidence in the significance of such experiments.

These results with RNA and DNA allow us to conclude that macromolecules can be purified away from their natural association with other molecules, injected free into the cytoplasm of eggs and oocytes, and still carry out some of their normal functions. These results, together with the evidence presented that injected molecules are not rapidly degraded or toxic, justify attempts to use injection experiments for investigating certain properties of injected molecules and recipient cells.

Specific applications of injection experiments

The following experiments have given results which would not have been predicted; they have been selected to show how the introduction of macromolecules into living cells can provide information not readily obtained by other means. The examples selected concern secondary modification of proteins, message stability, message-specific translational factors, and *in vivo* DNA polymerase assays.

Many kinds of proteins are secondarily modified after synthesis; molecular injection experiments have led to the conclusion that the enzymes involved are surprisingly general. Table 2 shows that frog egg or oocyte cytoplasm can modify correctly a range of proteins which they would never normally encounter. This lack of cell-type specificity of modifying enzyme systems suggests that the existence of modified pro-

teins in a cell is determined by the availability of amino acid sequences on which enzymes act and not by the availability of the enzymes themselves.

The stability of mRNA can vary substantially from one kind of message and cell type to another²¹, and message instability could be important for ensuring a changing population of new proteins as cells differentiate. The introduction of mRNA into a cell which is not synthesising that kind of mRNA provides a very sensitive test of mRNA stability under *in vivo* conditions. As seen in Fig. 2, α and β -globin mRNAs continue to be translated in oocytes for as long as 2 weeks, and without any obvious reduction in efficiency. Comparable experiments have been carried out in fertilised eggs²² and again the injected messages continued to be translated for as long as a week; by this time, over 15 cycles of cell division had been passed through, and the eggs had developed into swimming tadpoles, which were still synthesising rabbit haemoglobin. This remarkable stability of globin mRNA excludes the possibility that there is a general mechanism in oocytes or embryos which degrades all kinds of messages. Any natural message which is short lived in these embryos must evidently have RNase sensitivity coded into it, or must be specially complexed or compartmentalised in the cell.

Specialised cells are believed to contain molecules which enhance the translation of their characteristic messages²³. The effect of these message-specific factors is seen in cell-free systems. The unnatural composition of *in vitro* systems however, could result in a dependence on such factors in a way that would not be true of living cells. The use of injection experiments for investigating the role of message-specific factors and the control of translation in general demands careful quantitative control over the injection of nanolitre volumes of material. That this can be achieved in living cells is seen from the message saturation curves in Fig. 3. The injection of more than 10 ng of globin mRNA saturates the cell, and some component other than mRNA then limits the rate of its translation. Furthermore a message, once established, is displaced only slightly if at all by large amounts of other injected messages⁴⁹. This information makes it possible to carry out tests for the existence of message-specific factors by injecting mRNA into an oocyte which has previously been saturated with a different kind of mRNA. If the translation of a second message is unaffected by whether the cell has been previously saturated with the first message, this implies the existence of message-specific factors. Such experiments have been carried out in which globin and encephalomyocarditis (EMC)

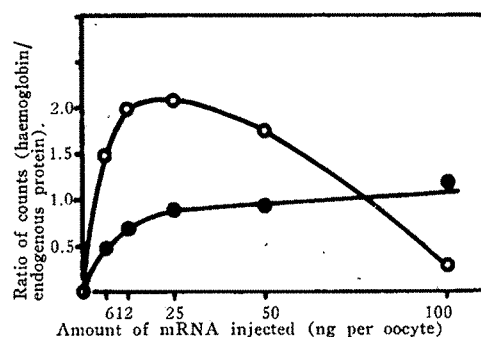


Fig. 3 Message saturation curves obtained by injecting increasing concentrations of mRNA or polysomes (for rabbit globin) into *Xenopus* oocytes. In calculating mRNA concentrations, it has been assumed that globin mRNA is 100% of the repurified 9S RNA of reticulocytes, and is 1.5% of the total RNA of polysomes. These experiments were carried out and analysed according to the procedures described in ref. 49. At least part of the difference in the saturation levels of polysomes (○) and pure mRNA (●) is due to the more efficient translation of α -globin mRNA in polysomes (which were in this case a crude preparation containing haemin)¹⁶. These are unpublished results obtained in collaboration with G. Marbaix.

TABLE 2 Secondary modifications of proteins in *Xenopus* oocytes

Protein	Modification in normal tissue of origin	Cell type in which protein is normally synthesised and modified	Evidence for modification in frog oocytes
α A2 crystallin	Acetylation of N-terminal methionine	Calf lens epithelium	N-acetyl methionine in N-terminal peptide ⁴⁵
Stable proteins of EMC virus	Cleavage of primary polypeptide	Mouse ascites cells	³⁵ S-methionine labelled virion proteins ¹⁷
Protamine	Phosphorylation	Trout testis	Phosphorylated peptides (unpublished work with G. Dixon)
Immunoglobulin light chain	Removal of ~15 amino acids from primary polypeptide	Mouse myeloma cells	Size of labelled light chains ^{46,47}
Collagen	Hydroxylation of proline	Cultured mammalian fibroblasts	Hydroxyproline in labelled protein ⁴⁸

Oocytes were injected with mRNA coding for the proteins specified, and then allowed to incorporate labelled amino acids. Labelled proteins were extracted and analysed. There are indirect, though strong, arguments against the possibility that the message samples contained contaminating messages for the modifying enzymes, as explained in references cited. This list includes all tests for secondary modifications so far completed.

virus RNA compete against each other. The translation of one of these messages is very largely inhibited by the injection of a saturating amount of the other, 24 h before (R. A. Laskey and J. B. G., unpublished). These two quite different messages seem to compete for the same translational components, and seem to be little affected by message-specific factors. This system can be applied to messages of different cell types once these have been purified to a level where they can saturate an oocyte's translational system.

The injection of mRNA into oocytes has been able to demonstrate a specific effect of haemin on the translation of α -globin mRNA. The background to this experiment is that rabbit or mouse α -globin mRNA is translated about five times less efficiently than β -globin mRNA in oocytes and eggs of *Xenopus*, though the α and β -globin mRNAs are translated with about equal efficiencies in mammalian reticulocyte cell-free systems¹⁶. If globin mRNA is injected at a subsaturating concentration, the addition of haemin raises the efficiency of α -globin mRNA translation in oocytes so that it is equal to that of β -globin²⁴. This effect shows that haemin behaves as a message-specific translational factor in oocytes, and it may well do so in normal reticulocytes. It is of interest that this specific effect of haemin has not been revealed in cell-free systems²⁶.

Experiments involving the injection of DNA have given the, at first sight surprising, result that active DNA polymerase and other replicative enzymes are present and potentially active in egg cytoplasm, DNA synthesis being stimulated by the injection of DNA into enucleated eggs¹⁸. Apparently egg cytoplasm is provided with a large store of DNA replicating machinery which is provided for nuclei during their enormously rapid rate of division during cleavage, when each S phase is completed in 10 min and mitoses occur every 20 min²⁰. It may well be difficult for cleaving embryos, which have small numbers of cells and therefore of genes, to synthesise new messages for all the proteins which are needed in rapidly increasing amounts at this stage. Presumably this is why egg cytoplasm contains a large store not only of active DNA polymerases, but also of histones²⁷, tRNA²⁰, RNA polymerases²⁹, and ribosomes^{28,30}.

Wider application of injection experiments

Molecular biology in living cells is unlikely to be of wide interest if it is restricted for technical reasons to Amphibia which have the advantage of large eggs, but the disadvantage of a long (~1 yr) life cycle. The same technique can however be applied to genetically more felicitous organisms such as *Drosophila* which has an egg nearly 100 times smaller than that of *Xenopus*, and even to the eggs of mice, with a volume 4,000 times smaller than those of *Xenopus*. Yet fertilised mouse eggs can withstand the injection of 20×10^{-12} l of oil droplets³¹ and normal mice have been born from eggs injected with 10^4 molecules of polyoma DNA (J. D. Bromhall, C. F. Graham, J. B. G. and R. A. Laskey, unpublished). It is even possible to inject single cultured cells³²

but with not more than 5×10^{-15} l. Evidently most animal cells can tolerate the injection of at least a few per cent of their own volume, and the introduction of very large numbers of macromolecules not normally present. It is obviously desirable, however, to use amphibian eggs or oocytes whenever possible for such experiments.

Prospects

The experiments described here have been primarily aimed at demonstrating that at least some kinds of purified macromolecules can carry out their normal function after introduction into living cells. What are the long-term prospects for such experiments? Three are worth mentioning. First, an assay system is provided in which the regulatory role of purified macromolecules can be tested in a living cell. It is certain that no cell-free system which might otherwise serve this purpose can reproduce exactly the conditions which exist in a living cell and which are, in any case, not accurately known. Unless the haemin effect referred to above turns out to be exceptional, results obtained from cell-free systems may not give an accurate indication of what would emerge from experiments with living cells.

The second long-term use for introducing macromolecules into living cells is connected with 'rescue' experiments. The injection of purified regulatory molecules, as outlined in the last paragraph, is applicable only when such molecules can be collected in sufficient quantity to be purified. It is very likely, however, that cells make use of regulatory macromolecules which are present only in very small numbers and which have not yet been identified. An attractive procedure for identifying these was devised by Briggs and his colleagues³³, and involves the rescue of embryos which are genetically or experimentally deficient in development. The first successful rescue experiment³⁴ achieved the restoration of germ cell growth and fertility to *Rana pipiens* embryos which had been ultraviolet-irradiated to destroy the germ plasm, a region of egg cytoplasm which is needed for normal gamete differentiation. Subsequently, experiments were carried out with a maternal lethal mutant in the *Azotol* in which eggs laid by a homozygous mutant (%) always cease development at the late blastula stage (even if fertilised by wild-type sperm³⁵). Some of these lethal embryos were rescued by injecting them as eggs with wild-type egg cytoplasm, so that development proceeded normally for several days beyond the usual stage of arrest³⁶. Some progress has been made in determining the nature of the corrective component^{37,38}. Since developmental arrest is caused in such cases by a single mendelian factor, this design of experiment could lead to the identification of a gene product which plays an essential role in early development. The success of this approach, however, depends heavily on the assumption that the gene product in question can retain biological activity when injected as a purified preparation into egg cytoplasm. The results, summarised here, with injected proteins and nucleic acids certainly give some encouragement for the long-term success of such an

approach. Clearly the isolation of appropriate maternal lethal mutants poses a formidable problem, though some of these have now been isolated in the *Axolotl*³⁰, and *Pleurodeles*⁴⁰; in *Drosophila*, where several suitable mutants are known⁴¹, a rescue experiment has been carried out with *deep orange*⁴².

The third long term use of molecular injection experiments concerns the arrangement of molecules within cells. The asymmetric distribution of molecules has for long been believed to be a primary cause of the first regional differences in developing embryos, and may well be involved in later stages of cell differentiation as well⁴. When, in time, some of these unequally distributed molecules have been identified, it will be possible to deposit samples of them in one region of an egg or other cell type. If the injected molecules remain localised at the site of injection, this will show whether their unequal distribution is causally connected with the divergent cell differentiation that subsequently takes place. If, however, the injected molecules are rearranged within the cell, this will open the way towards an experimental analysis of the cause of the intracellular localisation. The experiences with histones, referred to above, show that at least some kinds of injected proteins can be rapidly redistributed in a cell so as to take up their normal intracellular location.

I thank F. H. C. Crick, A. Colman, J. S. Knowland and H. R. Woodland for discussion of the content of this article.

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Building the Tower of Babble

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In remembering, without nostalgia, the dawn of molecular biology, Dr Chargaff deplores the loss of innocence that science has suffered in his lifetime.

I

"Two weeks later," Watson writes in his book *The Double Helix*, "Chargaff and I glanced at each other in Paris. A trace of a sardonic smile was all the recognition I got when we passed in the courtyard . . . of the Sorbonne" (page 132). Unfortunately retaining, as I do, only the trivialities of my

past, I remember the incident at the Biochemistry Congress in 1952 and the gawky young figure, so reminiscent of one of the apprentice cobblers out of Nestor's *Lumpazivagabundus*. I felt far from sardonic: I was looking for a toilet; but whatever door I opened, there was a lecture room and the same large portrait of Cardinal Richelieu.

"Sarcastic" or "sardonic" are the attributes usually accorded me in that book. But what I really was, when I first met the fervid pair in Cambridge, was baffled, for here were two people trying to fit the nucleotides into a helix and

worrying about its pitch—it became a double helix, I believe, after I had told them about our results—without bothering to look up the structures of the compounds they wanted to fit together. My dismay before so much unimpeded boldness will be understandable only to those who consider that at this time molecular biology did not yet exist. In the meantime the sciences have learned that it is profitable to transgress their boundaries and to over-reach their competence. Somehow they swallow more than they have bitten off. So perhaps 'laconic' would have been a better attribute, for what I jotted down when I left Cambridge was: "Two pitchmen in search of a helix."

II

It is, however, not of Watson's book that I want to write here; I have done that before¹. What I should like to do is to sketch in a few words how it felt to get into science, and especially into the field of nucleic acids, in those prehistoric times (nwc), before revelations had begun coming down from the mountains; before the 'invisible colleges' had begun to play their disagreeable roles as restrictive guilds; and, for that matter, before *Nature* had begun to sprout those sprightly little *feuilletons*. In addition, I should like to say a few words about the way in which the present scene strikes me.

No one who entered science within the past 30 years or so can imagine how small the scientific establishment then was. The selection process operated mainly through a form of an initial vow of poverty. Apart from industrial employment, important for a few scientific disciplines, such as chemistry, there were few university posts, and they were mostly ill-paid. One of my former chiefs at that time assured me that for him the opportunity of doing research as he pleased was sufficient recompense. (He had, besides, a comfortable private income.)

Science—or at any rate that part of science with which I am familiar—was small; it was cheap; it was wide open. One could still do experiments in the old fashioned sense of the word. Now, everybody is working away at 'projects' the outcome of which must be known in advance, since otherwise the inordinate financial investment could not be justified. Papers, however, continue being written in the old way, as if the discovery had come after the search. The number of very significant scientific discoveries made in the interval between the two world wars was truly enormous. The impulse persisted, or even increased, in the United States up to about 1950 or 1955, and then slackened perceptibly, almost in reverse proportion to the number of new scientists entering the several disciplines. I know of few instances in which the dialectical change of quantity into quality has been so obvious.

Since at that time it cost relatively little to perform scientific experiments, it was always possible and even inviting to venture into fresh areas. The risk was minimal and so, unfortunately, sometimes were the results. But the road to them was always delightful. This changed gradually as a result of several powerful technological advances. The introduction of isotopic labels gave rise to an industry whose products became increasingly more expensive, as the variety of the compounds grew and purity standards declined. The construction of powerful centrifuges and other physical equipment extended immensely the range of the possible, but even more the cost of achieving it. Other advances, especially in chromatography, electrophoresis, and spectrophotometry, contributed more than they took away. Still, it remains that according to my highly unreliable estimate a current paper of mine costs about 20 to 25 times more to produce than an equivalent article 35 years ago, if such things can be weighed and compared. The argument that such calculations are meaningless—for what would we give if we had one more Mozart opera—can be rejected; none of us writes Mozart operas.

III

The small number of scientific workers engaged in research had other consequences. It was easy to open new fields and to go on cultivating them; there was no fear of immediate dispossession as is bound to happen now. There were relatively few symposia, and those that existed were not attended almost exclusively by hungry locusts yearning for fields to invade. Bibliographies were comparatively honest, whereas now entire packages of references are being lifted by a form of transduction, as it were, from one paper to the next; so that if some work gets into the habit of not being quoted, it never will be so again. The break in the continuity of the tradition has, perhaps, been one of the most disastrous effects of the scientific mass society in which we are living now.

The illusion that what is new is true has distorted the very sense of scientific research. The urge to be 'with it' is incompatible with the search for truth about nature—and that is what science is—and where one can say 'this is no longer true,' nothing is true. Some years ago, I heard an eminent colleague declare at a congress: "The results that I reported last year were based on facts that are no longer available"—a form of recantation that should have delighted Galilei without offending the Inquisition. Our current literature is brimming with facts, but many, I am afraid, are no longer available. If the vaunted self-purification of science has broken down some time ago, this is only in part a result of the ever increasing complexity of ever more poorly described experiments. It is even more a consequence of the pressed and driven mood in which research often is performed now: "... in a hurry of waste, and haste, and glare, and gloss, and glitter."²

Two more points will complete this hasty sketch of a Golden Age that never was. As the number of scientists was so small, it was easy for a young man to establish himself. Two or three decent papers, and he was in, for what it was worth. Another consequence of the restricted dimensions of our scientific knowledge at that time, before it was overwhelmed by multiple massive explosions of facts, many of them of the utmost triviality, was that it was still possible to comprehend the essentials of one or even of several sciences. This bucolic security has, I believe, ended: "out of swimmers we have all turned into floaters."³ Or, to put it less metaphorically: "the sciences, like other professions, cannot endure if their practitioners are unable to know more than an ever-smaller portion of what they must know in order to function properly."⁴ Even granting all the present difficulties in acquiring sufficient knowledge, I must say that the extreme dislike, and therefore ignorance, of chemistry I have often encountered among molecular biologists is puzzling. Chemistry is the science of substances; and to the extent that molecular biology deals with substances and not in them, as if they were commodities, a thorough acquaintance with chemistry is advisable.

IV

I should be sorry if I give the impression that I am trying to paint an *aurea aetas*. I grew up in bestial times, and they have become worse. I have written elsewhere of my surprise that such bad times gave rise to so much good science^{5,6}; probably the only activity of the human mind that has, until recently, been in the ascendant. It is, however, not astonishing that in a rotten society even the saints will carry a slight aroma of rottenness.

One of the outstanding curses of my lifetime has been the manipulation of mankind by advertising and propaganda. In the sciences this evil force had for a long time remained unnoticeable, perhaps because expanding capitalism and youthful imperialism had other worries, perhaps because scientists owing to their small number survived unharmed in the crevices of a society that still paid little attention to

them. The first figure of the highest rank that I saw being lifted by the wheels of the publicity machine and turned into a celebrity was Einstein. (Einstein's very interesting correspondence with Max Born and with Mrs Born displays many instances of this process; compare, for instance, the letters of October 7 and 13, 1920, pages 62 and 65.) Freud, who could have been another victim, escaped the glare entirely, being 23 years older. He also benefited from the fact that his discoveries were relatively accessible: an element of impenetrability helps. There may have been before my time another instance of mindless acclaim, namely, in the case of the Curies and the discovery of radium, to judge at any rate from the slightly paranoiac image reflected in Strindberg's *Blue Books*; but then one could hardly yet have spoken of the mass media.

When molecular biology appeared on the scene, however, the publicity machines were all in position and it was time for the saturnalia to begin in full force.

V

I should not want to leave the impression that molecular biology began with the double helix. The reason, and even the date, of the appearance of sectarian movements is hard to determine. I have, in another article, attempted to delineate its pedigree which probably had its origin in the discovery of the transforming properties of DNA and the introduction of bacteriophages as objects of biological research^{5,6}. In my opinion, there really was little sense in creating a new science that consisted essentially in the application of chemistry, and to a limited extent of physics, to biology; that is what biochemistry and biophysics stand for.

I remember vividly my first impression when I saw the two notes on DNA that appeared in *Nature* 21 years ago^{8,9}. The tone was certainly unusual: somehow oracular and imperious, almost decalogous. Difficulties, such as the even now not well-understood manner of unwinding the huge bihelical structures under the conditions of the living cell, were brushed aside, in the Mr Fix-it spirit that was later to become so evident in our scientific literature. It was the same spirit that soon after brought us the 'Central Dogma' to which I believe I was the first to register my objection, never having been very fond of gurus with a PhD. I could see that this was the dawn of something new: a sort of normative biology that commanded nature to behave in accordance with the models.

The structural model proposed for DNA in the first note⁸, a helical dyad held together by base pairing, seemed to me not only an aesthetically pleasing solution, but also the most plausible inference from the base-pairing regularities earlier discovered by us in many DNA preparations¹⁰. I was much less in agreement with the scheme for DNA replication proposed in the second note⁹; and even now, 20 years and thousands of experiments later, I cannot say that I am reconciled with it completely, the mechanism of DNA synthesis *in vivo* still being obscure to me.

I do not know whether in 1865, when Kekulé put forward the structural model of benzene, which was to revolutionise organic chemistry, neckties appeared forthwith, embroidered with the pretty hexagons. I should rather doubt it, since at that time mass cretinisation had not yet begun and advertising still was a home industry. At any rate, the publicity carnival that ensued upon the unveiling of the DNA model was probably unique in the history of science. I have given a brief description in my Bertner Foundation Award Lecture¹¹.

VI

Scientific induction is actually the resultant of a parallelogram of rational and irrational forces. That is why in many respects Science is not a science, it is an art. The importance

for scientific research of imagination, of unpredictable conclusions from unexpected analogies, can, therefore, not be overestimated. If all can be declared in advance, then there remains only the dull verification. The greater the reliance on axiomatic constructions, on prescriptive models must be, the more restricted becomes the freedom of the scientific intellect, the narrower is the range of what can be discovered. These are, I am afraid, the conditions under which a great part of molecular biology now operates.

Research always runs the danger of overasserting the truth of its observations, leaving even less space for dialectics to turn around. For me, however, scientific truth consists of what has not yet been disproved; it is at best a dense mosaic of approximations. Therefore the chase is worth so much more than the prey; or, to put it less violently, the road counts more than the destination. Do I hereby propose Sisyphus as the patron saint of the scientist? Not in a general way. What was tragic about the fate of this mythological celebrity was that he was always lifting and losing the same rock; which is, indeed, what many molecular biologists are now doing.

Many of the papers in this field are very competent technically. Since the same procedures are used, regardless of the particular biological system investigated, the results usually confirm each other; and this is taken to prove the unity of nature. When novel apparatus and techniques are introduced, a new set of results is obtained; and this is registered as scientific progress. A pall of monotony has descended on what used to be the liveliest and most attractive of all scientific professions. The noble study of botany, for instance, has been all but banished from many universities. Before, the biological sciences had each their characteristic faces and their distinct spheres of interest into which they drew different types of scientists. Now, when I go through a laboratory, be it of virology or of developmental physiology, there they all sit before the same high-speed centrifuges or scintillation counters producing the same superposable graphs. There has been very little room left for the all-important play of scientific imagination. *Homo ludens* has been overcome by the seriousness of corporate finances.

VII

Our period, just because it is altogether so weak intellectually, is given to extraordinarily strong assertions. Many of the great constructions of our time—existentialism, structuralism, transformational grammar, the central dogma and some other sloganised tenets of molecular biology—have all looked, from their very beginning, somehow shoddy and overblown. There was about them a flavour of not being entirely earned, as of trick images viewed in a mirror. As the mirror clouded over, the images vanished. Much of what they claimed may actually have been true; but they looked like packages much too large for what they contained. One got the impression that it was often the wrapping that produced the particular content; just as there are now packaging artists who wrap entire mountains in plastic flimsy.

This is not the note, however, on which I wish to end. I should like to recall a few names. They are the names of those who had done some of the basic work on nucleic acids and whom I got to know personally, either before or after I left sweeter fields to concern myself with the harsh problems of nucleic acid chemistry. T. B. Johnson, of Yale University, who got me first to America, did some of the most important work on the chemistry of the purines and pyrimidines. Steudel, once one of Kossel's collaborators, I met at the University of Berlin. Alexander Todd showed me through the organic chemistry laboratories when I visited Oxford in 1934. I often saw P. A. Levene at the Rockefeller Institute; his work, especially on the sugar constituents of the nucleic acids, deserves more admiration than it now receives. At

the same institute I sometimes got a glimpse of the great and modest Avery. And there were Gulland and Jordan and J. N. Davidson, Brachet and Caspersson, Bawden and Pirie, Hammarsten and Jorpes, Thannhauser and Gerhard Schmidt, Mirsky and Pollister, and the gentle and courteous Belozersky in Moscow. Zacharias Dische, without whose diphenylamine reaction most of the work on DNA could not have been performed, has for years been a colleague at Columbia University. Many of these men have gone, but the list is, luckily, not entirely sepulchral. They all did their work before the strip-mining of nature had become so prevalent, before researchers had become alienated from the objects of their study. In the tower of forlornness, which the House of Science has become in my time, the inhabitants all speak the same language, but do not understand each other.

Few will be of my opinion, certainly not the one who made fun of me some time ago in a magazine article, saying

that my ideal of a scientist was Louis Pasteur played by Paul Muni. This may be so, though I doubt it. What I do dislike, however, is to see *E. coli* impersonating nature. The difference in talents is really too great.

- ¹ Chargaff, E., *Science*, **159**, 1448 (1968).
- ² Byron, Lord, *Don Juan*, **10**, 26.
- ³ Chargaff, E., *Essays on Nucleic Acids*, 109 (Elsevier, Amsterdam, London, New York, 1963).
- ⁴ Chargaff, E., *Perspect. Biol. Med.*, **16**, 486 (1973).
- ⁵ Chargaff, E., *Experientia*, **26**, 810 (1970).
- ⁶ Chargaff, E., *Science*, **172**, 637 (1971).
- ⁷ Einstein, A., Born, H., and Born, M., *Briefwechsel 1916-1955* (Nymphenburger Verlagshandlung, Munich, 1969).
- ⁸ Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737 (1953).
- ⁹ Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 964 (1953).
- ¹⁰ Chargaff, E., *Experientia*, **6**, 201 (1950).
- ¹¹ Chargaff, E., in *Developmental and Metabolic Control Mechanisms and Neoplasia*, 7 (Williams and Wilkins, Baltimore, 1965).

Molecular biology and metaphysics

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"And now the announcement of Watson and Crick about DNA. This is for me the real proof of the existence of God". (Salvador Dali, 1964).

FRANCIS Crick prefaces his 1966 John Danz Lecture *The Nature of Vitalism*¹ with this quotation from Dali, but mentions neither the surrealist painter nor God in the body of his text. Why, then, does Crick quote Dali? As readers of *The Double Helix* know, James Watson has "never seen Francis Crick in a modest mood". But not even Watson would claim that Crick really believes that they have delivered the final proof for the existence of God. No, my friend Crick finds Dali's statement a tremendous joke and though Dali's intent was surely serious, Crick is making fun of him by according Dali the place of honour under the masthead of an antireligious tract. But I think Dali has sized up the situation quite correctly: the achievements of molecular biology have furnished proof for the existence of God. And that, as I shall try to show here, could be bad news.

To begin, I draw attention to the fact, long appreciated by philosophers, that science rests on metaphysics. That is to say, the attempt of science to explain the world is based on a set of *a priori* beliefs whose validity cannot itself be the subject of scientific enquiry. Now that the once-fashionable philosophy of positivism (which threw metaphysics into the philosophical ashcan) is all but moribund, it should hardly be necessary to belabour this point. But since in *Chance and Necessity*², the best known philosophical book by a molecular biologist, Jacques Monod holds fast to the 17th century empiricist notion of a science free of subjective projections into nature, which Kant had laid to rest nearly 200 years ago, a reminder of the transcendental basis of science may not be out of place.

Platonic doctrine

One of the most fundamental of the *a priori* beliefs that makes science possible is Plato's monistic doctrine of a single principle that not only regulates the course of the sun and the stars but also prescribes to all creatures their proper behaviour. That single principle is God, or his atheistic synonym, Eternal Reason, whose power, according to Isaiah Berlin, "has endowed all things and creatures each with a

specific function; these functions are elements in a single harmonious whole and are intelligible in terms of it alone. . . . This unifying monistic pattern is at the very heart of traditional rationalism, religious and atheistic, metaphysical and scientific, transcendental and naturalistic, which has been characteristic of Western civilization. It is this rock upon which Western beliefs and lives have been founded. . . .³ Thus belief in God is not only not incompatible with rational thought but it is the metaphysical axiom from which it follows that an explanation of the world is accessible to human reason. This accessibility is, in turn, provided for by the existence of God-given Natural Law that governs the orderly operation of the world. Thus a scientist is a man who believes in God, for without this belief it would be futile to try to discover His Laws.

I will document this allegation, which the majority of contemporary scientists surely reject, by quasi-humorous citations from two great scientists, since according to Freud, jokes reveal repressed thought⁴. First, in his Danz Lecture, Francis Crick points out that though the three-dimensional conformation of proteins can, in principle, be worked out from the structure of their component amino acids, the necessary computations are almost prohibitively long. But proteins find their conformations all the same because "Nature's own analogue computer—the system itself—works so fantastically fast. Also she knows the rules more precisely than we do. But we still hope, if not to beat her at her game, at least to understand her". Second, as is well known, Einstein affirmed his unwillingness to accept the epistemological implications of Heisenberg's Uncertainty Principle by saying that "God does not play at dice". By these statements, Crick and Einstein reveal their allegiance to the Platonic doctrine, and Crick probably makes the verbal substitution of a personified 'Nature' for 'God' only to avoid giving the impression that he is a Christian.

The application of the Platonic doctrine is not limited to science, of course, but also provides the foundation for Western ethics. These ethics, held by Judeo-Christians and Western atheists alike, envisage the existence for their own sake, of objectively valid, ultimate values of right and wrong. It is because of the recognition of these God-given (or 'natural') values that we can speak of crimes, or morally justify or condemn any act. But as Berlin points out, there is also another system of ethics, which Berlin terms 'pagan'

and which derives its authority, not from God but from the obvious fact that man is a social animal who lives in communities. In the pagan system there are no ultimate values, no intrinsic rights or wrongs, only communal purpose, and so moral judgements here are relative rather than absolute. Since, our adherence to the Platonic ideal notwithstanding, we cannot ignore the pagan reality, the ensemble of Western aims and values is internally inconsistent. Berlin credits Machiavelli with the discovery of this fact and with showing "that the belief that the correct, objectively valid, solution to the question of how men should live can in principle be discovered is itself in principle, not true". According to Berlin, it is for having split open the Platonic rock upon which Western lives are founded, for pointing out the impossibility of the City of God, rather than for being 'Machiavellian', that Machiavelli has drawn an ecumenical and everlasting hatred from men representing the whole spectrum of Western religious, philosophical and political thought.

Western contradictions

What can be done to resolve this contradiction in Western lives? Admittedly, in the ethical domain one may reasonably doubt whether grafting the lofty ultimate values of Judeo-Christianity on pagan down-to-earth ethics has really been a good thing. But in the scientific domain the notion of Natural Law has evidently been gloriously successful. Particularly since Galileo, modern science has gone a long way in showing that reason can explain the world and that by the understanding thus obtained one can gain mastery over Nature. So how can we give up our Platonic legacy and return to uncorrupted paganism? It is in this light that we must view the triumph of molecular biology over vitalism that both Crick and Monod celebrate in their essays.

Crick has "a strong suspicion that it is the Christians, and the Catholics in particular, who write as vitalists, and it is the agnostics and atheists who are antivitalists". Crick may be right in his suspicions, but I see no philosophical or theological connection between Christianity and vitalism. On the contrary, in so far as vitalism boils down to the proposition that a full explanation of life is beyond the capacity of the human intellect, it subverts the Platonic doctrine. By questioning the accessibility of the world to reason, vitalism raises doubts about the existence of God as the author of Natural Law. Accordingly, the writings of the Jesuit Teilhard de Chardin, a source of inspiration for many latter-day vitalists, were considered heretical by his superiors in the Society of Jesus and suppressed by them. Thus, as long as Schrödinger's query "What is Life?"⁵ had not been answered and as long as science had not been able to account for the basic processes that distinguish living matter from dead, there still seemed a chance that life is not God's handiwork after all. But as Dali recognised, now that molecular biology has shown how life can be explained in terms of ordinary physics and chemistry, further proof has been delivered that God designed the world and saw to it that His plans are comprehensible to man. In other words, by contributing to the validation of the monistic doctrine, Watson and Crick have made it more difficult for Western man to get off his Platonic rock.

The achievements of contemporary biology have helped to bring to light the moral contradictions inherent in the Western ethical system. To appreciate the nature of these contradictions we must consider the concept that is altogether central to that system, namely the soul⁶. Belief in the soul has been as essential for Western ethics as belief in Natural Law has been for Western science. As is well known, the modern formulation of the problem of the soul is due to Descartes, who laid the philosophical foundations for physiology, and thus also for molecular biology, by advancing the fruitful notion that the bodies of humans and animals can be regarded as machines. But since moral principles

obviously do not apply to machines and do apply to humans, humans must be more than automata in human shape. The extra something that makes humans more than automata is the soul. It is from his incorporeal soul that man derives both the freedom of and the responsibility for action, without belief in which there can be no morality. For dealing with the intersection of morals and human biology, nothing has thus far replaced the Cartesian body-soul dualism. Admittedly, as Crick points out, "many scientists believe that the soul is imaginary and that what we call our minds is simply a way of talking about the function of our brains". But in denying their belief in the soul, these scientists, in so far as they are moral beings, merely resemble Molière's Monsieur Jourdain, who did not realise that he was speaking prose. It must be noted that Monod is not one of these scientists, since he devotes a major part of *Chance and Necessity* to showing just why what he calls "the modern soul" is in distress.

Mental illness

Consideration of two examples of a contemporary conflict between biology and morals show that this conflict arises from the impossibility of resolving the body-soul dualism. One of these examples is provided by radical criticism directed against the treatment of the insane, indeed against the very concept of insanity. In an essay, *Mental Illness as a Metaphor*⁷, Thomas S. Szasz, himself a professor of psychiatry, argues that mental illnesses are not genuine diseases and that psychiatry is not a *bona fide* medical specialty. One of the two main arguments put forward by Szasz in support of this claim is that a medical patient can only be a person who voluntarily assumes that role and a physician can only be a person who gives treatment with the consent of his patient. Since according to Szasz, psychiatric treatment is, more often than not, involuntary, insane persons are not really ill and psychiatrists are not really physicians. Thus psychiatric practice maintains not health but law and order, and does so in a manner which is incompatible with the ideals of a free and just society. Szasz' other main argument is that insanity is not attributable to "an abnormality or malfunctioning of [the] body. . . . Strictly speaking. . . . disease and illness can affect only the body. Hence there can be no such thing as mental illness. The term 'mental illness' is a metaphor". Thus, in contrast to Crick's "many scientists" who believe "that what we call our minds is simply a way of talking about the function of our brains", Szasz has recognised the unacceptable moral consequences of that belief. It is a "systematic strategy . . . for stigmatizing and depersonalizing persons". Evidently, Szasz is informed by the notion that the "real" person, the free and responsible agency, is not the body but the incorporeal soul. Thus, to treat insane people as if they were sick is, according to Szasz, to confuse medicine with morals. In other words, the biological reification of the soul, the dissolution of the Cartesian dualism, is incompatible with the maintenance of Western ethics.

Genetic engineering

The second example of a contemporary conflict between biology and morals is provided by the imminent extension of 'genetic engineering' to humans. Though the proponents of a deliberate manipulation of man's genotype intend it in all sincerity to augment human welfare, this prospect is generally viewed with extreme distaste, and not only by the righteous guardians of so-called 'social responsibility' in science. Why should an application of the fruits of biological research of overt philanthropic intent, which is not meant to kill defenceless civilians, exploit subject peoples, or despoil the Earth, nevertheless evoke the spectre of Dr Strangelove? To fathom the root of this problem we may consider a

fantastic facet of genetic engineering which, though it seems taken straight from the pages of Aldous Huxley's *Brave New World*, is actually likely to become a practical reality before long. This is the asexual reproduction, or cloning of humans by transfer of somatic diploid nuclei from a male or female donor to enucleated eggs. Out of such eggs can develop a clone of genetically identical individuals, all of exactly the same hereditary constitution as the donor. This technique will soon make it feasible to abandon the old-fashioned genetic roulette of sexual reproduction and start populating the Earth with identical replicas of carefully chosen, ideal human genotypes. As far as I know, no one has actually come forward to advocate the institution of this programme, even though from a eugenic point of view, it offers tremendous possibilities. Evidently, the idea of cloning humans is morally and aesthetically completely unacceptable. But why is it that whereas it would be fun to have Kant, Beethoven, Isadora Duncan, Einstein, Clark Gable and Marilyn Monroe living on your block, the thought of having hundreds or thousands of their replicas in town is a nightmare? The reason for this horror is the generally shared belief in the uniqueness of the soul. Even though the soul is incorporeal, it is supposed to fit the body, and hence it is not conceivable that a unique soul inhabits each of thousands of identical bodies. In other words, a human clone would not consist of real persons but merely of Cartesian automata in human shape.

To oppose human cloning, however, is to betray the Western dream of the City of God. All utopian visionaries, from Thomas More to Karl Marx, think of their perfect societies as being populated not by men but by angels that embody all of the best and none of the worst human attributes. As long as, due to the vagaries of the sexual reproductive mode, there was not the slightest chance that such angelic populations could actually arise, this seemed like a believable dream, a hope for the future. But now, when advances in molecular and developmental biology have brought asexual generation of homogenous populations selected for angelic properties within practical reach, it suddenly becomes clear that this is not the perfect society that is wanted after all. What is wanted is the impossible: a perfect society made up of a motley collection of imperfect, unique souls, warts and all.

Chinese alternatives

These conflicts are unlikely to be resolved within the context of the Western tradition. What it would take to remove them from Western lives is to abandon belief in God, His ultimate values and His Natural Law. But is it possible to run a civilised society on purely pagan metaphysics? It certainly is, since another great civilisation, the Chinese, has operated for millennia on just that basis. Chinese lives are not founded on the Platonic rock that Machiavelli split open and when viewed in the light of the Chinese tradition, Western scientific atheism is merely old divine wine in new bottles. Just about the time when the Platonic doctrine was formalised in Greece there developed in China the two complementary philosophical-ethical systems of Taoism and Confucianism which have governed life in the Middle Kingdom ever since. The precepts of Confucianism are based on the premise that man is a social creature and that therefore there is virtue in harmonious social relations. These relations are made harmonious not by obedience to universally valid abstract moral principles but by exact adherence to a combination of prescribed etiquette and ritual. Taoism, is a transcendental moral philosophy whose main relevance is for the inner life rather than for social relations. Its precepts are based on the belief that man is part of (non-personalised) Nature and that, therefore, his life must take the path, or *tao*, of natural events. Man, following the *tao*, must abjure all striving,

distrust reason, and attempt to attain a state in which he is as free from desire and sensory experiences as possible. Neither Confucianism nor Taoism invokes God or Eternal Reason as the source of its authority, nor do they posit the existence of Natural Law or ultimate values. Rather, both systems endeavour to provide for man's harmony with his environment.

In my opinion it is highly significant that Chinese attitudes are now coming to exert an ever-growing influence in the West. This influence is no longer confined, as it was only a few years ago, to Zen Beatniks, New Left Maoists, transcendental meditation freaks and other far-out members of the Counterculture. Instead, it has reached the very Pillars of Society. For instance, the recent, mainly middle-class, interest in 'ecology' is a radical departure from the ancient Western aim of dominating Nature. It is nothing less than a Taoist subversion of the hallowed 19th century belief in progress. Similarly, the recent accommodation of the two Superpowers, the United States and the Soviet Union, and the ending of the quarter-century-long Cold War, suddenly abandons, without any profound political change in either country, the righteous reciprocal Crusade to smite the Anti-christ or Enemy of Man. It constitutes a Confucian subversion of the Western romantic ethic of the Nation as the Protector of the True Faith and places harmony above ideology in international relations.

Another important consequence of the Sinification of the West is the recent, and for most old-time scientists quite incomprehensible, loss of faith in the 16th century creed of Francis Bacon that the discoveries of science ameliorate the human condition. More and more people have come to embrace the belief that what it will take to save the world is not to build a better mousetrap but to understand man. But with this development we are reaching the limits of biology: whereas its methods are evidently capable of giving a satisfactory account of human physiology, human psychology does not seem to be accessible to the procedures discovered by Galileo. This conclusion follows from the growing ascendancy of the 'structuralist' over the 'behaviourist' approach to the study of human behaviour. Behaviourism, whose working methods are more or less those of modern science and whose propositions are therefore subject to validation, has thus far produced mainly trivialities. Structuralism, on the other hand, particularly as pioneered by Freud, has offered some deep insights. But structuralist methodology, and its central theoretical axiom that the causal relations governing overt behaviour occur between covert 'deep' mental events whose existence can be inferred only indirectly, renders scientific validation of these insights well-nigh impossible.

Thus the metaphysical implications of modern biology are not quite what my friends make them out to be. It is not the triumph of molecular biology over vitalism that has made belief in God unnecessary. Rather, it is the apparent inaccessibility of the human psyche to scientific study and the prospect that psychoanalysis (which Crick regards as "mental bleeding") may be the best we can do that still leaves open the possibility of abandoning Him. Therefore, not because of but despite "the announcement of Watson and Crick about DNA", the West may still remain free to descend from its Platonic rock and join the East in a pagan One World.

¹ Crick, F., *Of Molecules and Men* (University of Washington Press, Seattle and London, 1966).

² Monod, J., *Chance and Necessity* (Knopf, New York, 1971).

³ Berlin, I., *The New York Review*, November 4, 20-32 (1971).

⁴ Freud, S., *Der Witz und seine Beziehung zum Unbewussten*. (Fischer Taschenbuch Verlag, Frankfurt-am-Main, 1958).

⁵ Schrödinger, E., *What Is Life?* (Cambridge University Press, New York, 1945).

⁶ Flew, A., *Immortality in Encyclopedia of Philosophy* (edit. by Paul Edwards), 4, 139-150 (Macmillan, New York, 1967).

⁷ Szasz, T. S., *Nature*, 242, 305-307 (1973).

DNA before Watson—Crick

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"Nucleic acids bulk so large in the composition of cells that it seems certain that these substances are involved in some important biological functions. Nevertheless, with one exception [bacterial transformation], there has been no proof of a specific biological function mediated by one of these polynucleotides; no enzyme, hormone, vitamin, or even vague 'growth substance' has been found to be a nucleic acid."

WITH these words Pollister, Swift and Alfert introduced their work on the metabolism of DNA to a conference on the biochemistry of nucleic acids, held at Oak Ridge in April 1950. Their paper furnishes the historian with clear evidence that by this date DNA was receiving serious consideration as a major component of the hereditary code-script, quite independently of the work on the biology of bacteriophages and on the base composition of DNAs. When Watson and Crick turned to the structure of DNA they knew it was a macromolecular compound, a major constituent of the sperm nucleus, phage head, and chromosome. They could not but be aware of the association between the staining properties of chromatin and of DNA. Only extreme ignorance would have prevented them from knowing of the correlation between chromosome duplication and the synthesis of DNA. Add to these facts the proven identity of the transforming principle with DNA, and one is presented with compelling reasons for identifying DNA with the genetic substance in October 1951 when Watson came to Cambridge and began his collaboration with Crick. Or is the situation only seen as compelling with the aid of hindsight? We must recall that the earlier decision against the genetic role of DNA had to be overcome. It was not so easy in the 1940s as it may seem now to transfer the onus for proof of genetic function from DNA to protein. On what grounds had the candidacy of DNA been rejected and how was the evidence, so damaging to DNA, undermined?

Looking for the hereditary substance

At the end of his life the Swiss physiologist, Fritz Miescher, arrived at the figure of 80% for the nucleoprotein content of the sperm head. His results were reinterpreted by Oswald Schmiederberg who gave the figure of 96% (ref. 2). Those who accepted that hereditary transmission is due to chemical compounds were therefore anxious to discover in nucleic acids and protamines evidence of chemical differences. They looked for metabolic stability and for continuity from one cell generation to another, but they did not find it! Miescher doubted the importance of nucleoprotein. He had high hopes of a mysterious iron-containing compound which he called "karyogen" supposedly located in the centre of the sperm head, where it was wrapped around with nucleoprotein. His successors, Burian, Mathew, Boveri, Loeb and Strasburger were by no means convinced that either nucleic acid or protamine constituted the hereditary substance. Even the American cytologist, E. B. Wilson, often cited as a supporter of the DNA theory of the gene, in fact identified the genetic substance as 'nuclein', a chemical

compound of nucleic acid and protein. Some authorities regarded mere chemical compounds inadequate for hereditary transmission and invoked 'higher' morphological structures. In this they were supported by the supposed discovery of a cycle of loss and recharging of the chromosomes with nucleic acid. When the cell divided, the chromosomes became short and fat; they took up basic stains avidly and showed up clearly under the microscope. After division these same chromosomes elongated and their power to attract basic stains weakened. The staining substance—chromatin—or DNA, was not, it seemed, the stable continuing genetic material. This must be "achromatin", possibly the protein fraction of the chromosome.

In the sperm head, protein was present in the form of rather specialised compounds known as "protamines". They were found to be much simpler in their composition than any other class of proteins, some having as much as 87% by weight of one amino acid, arginine. Moreover, no continuity could be traced between them and the histone-type of protein of normal body cells. Yet this did not dissuade some biochemists from seeking genetic specificity in the protamines. Thus in 1935 Waldschmidt-Leitz saw the structural comparisons of protamines as preparing "the ground for a chemical investigation of problems of heredity"³. In 1950 K. Felix in Strasbourg was applying paper chromatography to the protamines to determine the quantities of the six mono-amino acids which made up one tenth by weight of the fish protamine, clupein⁴. In 1952 his study of the breakdown products of clupein gave over one-third in the form of four arginine residues strung together, surely reason enough to pass some adverse comment on the potential variety of protamine molecules? No such remark was made⁵.

Others, doubting the potential of both nucleic acids and protamines, searched for other compounds in nuclei. Edgar and Ellen Stedman found only 70% of the sperm head in the form of nucleoprotein; the remaining 30% they attributed to proteins higher than the protamines and histones, which they called 'chromosomin'. This, they claimed was a group of proteins, the amino acid content of which differed from species to species. Now they could overcome the failure of chemistry to provide the required chemical differences by identifying the hereditary materials with chromosomin. Nucleoprotein was no more than a constituent of nuclear sap! To be sure it was condensed on the chromosomes dur-

TABLE 1 Base analyses of nucleic acids from 1902 to 1950

Date	Molar ratios				Authors	Nucleic Acid
	A	G	C	T/U		
1902	1.0(?)	0.98	—	1.17	Osborne and Harris	RNA
1905	0.47	0.23	1.35	1.0	Levene	DNA
1906	1.30	0.98	0.63	1.09	Steudel	DNA
1908	0.98	0.98	1.02	1.02	Levene and Mandel	
1909	1.0	1.3	1.7	?	Levene	RNA
1947	0.8-0.9	1.0	1.1-1.2	1.0	Gulland	DNA
1948	0.8	2.0	1.0	0.25	Chargaff	RNA
1949	1.6	1.3	1.0	1.5	Chargaff	DNA
1950	0.29	0.18	0.18	0.31	Chargaff	DNA

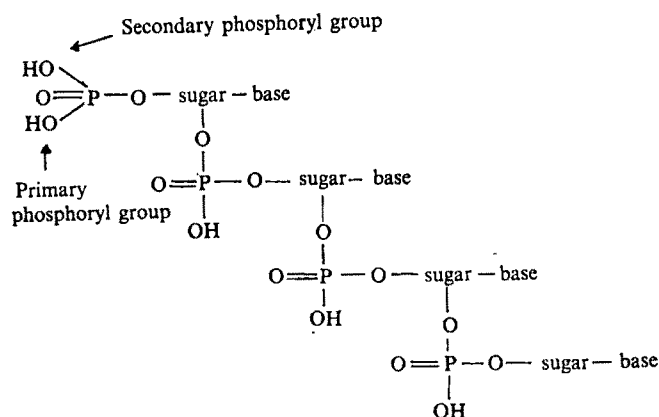


FIG. 1 The tetranucleotide according to Levene.

ing certain phases in the nuclear cycle—as in the sperm head—but this was a transitory state of affairs⁹.

In 1946 Mirsky and Pollister applied their salt extraction technique to trout sperm. The nucleoprotein content of the sperm head came to 91%, leaving 9% of what they called a 'residual protein'. This was distinct from protamine, and like chromosomin, it seemed a promising candidate for the hereditary substance. Not until 1951 did Felix put his authority clearly behind the conclusion that the head of the sperm contains only nucleoprotamine⁸. This search for a residual protein indicates that DNA itself was unacceptable as the genetic material. Although the achromatin hypothesis played a part in achieving this situation it was the tetranucleotide hypothesis which increasingly threw DNA into the shade.

The tetranucleotide hypothesis (or theory as Levene called it) asserted that DNA and RNA had a molecular weight of about 1,300, and contained one of each of the four bases (adenine, guanine, cytosine and thymine in DNA; adenine, guanine, cytosine and uracil in RNA). Because these bases were found to be present in the form of nucleotides (sugar-phosphate + base) the molecule was called a tetranucleotide. Early analyses had shown there to be four phosphorus atoms per molecule, and when four bases were discovered in DNA it was natural to hope to find them in equimolar proportions. The fact that base analyses (see Table 1) deviated by as much as 54% from expectation, and were not always complete, did not trouble these investigators. They had a difficult task on their hands; powerful methods were needed to split the molecule, and bases were easily lost or altered. Osborne and Harris began the tradition of equimolar proportions; Steudel extended it and Levene established it. In the process he twice quoted the figures which Steudel had predicted from the tetranucleotide as his experimental results! Since Levene identified esters of the pyrimidines with two phosphoryl groups attached to them in the breakdown products of DNA he developed an argument for the alternating sequence—pyrimidine, purine, pyrimidine, purine—and rejected any other arrangement. Arguments such as this depended, of course, on the assumption that DNA was a small molecule.

Yet the tetranucleotide hypothesis was not swept away once the macromolecular nature of DNA became established in the 1940s. Doubtless this was to a large extent a case of the entrenchment of a theory. In part it must also have been due to the great success of the theory, for it represented the conclusion of Levene's brilliant work on the constitution of nucleic acids. Out of a confusion of erroneous and vague ideas he had extricated a clear concept—the equimolar proportions of the bases—and built upon it the model of the nucleotide which by sugar-phosphate linkage gave the same chain structure that we accept today (Fig. 1). This hypothesis was, then, a remarkable case of a paradigm which started off as a work-

ing hypothesis, gained explanatory power when associated with the nucleotides, and became so rooted in the chemical thought of nucleic acid chemistry that a revolution was required to overthrow it.

Early estimates of the molecular weight of DNA were based on percentage composition, but although this yielded only the minimum value, it was universally taken to be the true molecular weight—about 1,300. In the nineteenth century Miescher had shown DNA to be a slowly diffusing compound, and Neumann had perceived the distinction between an extract of DNA which readily formed a gel and one which did not. The latter, he thought, resulted from the depolymerisation of the former. But as Fig. 2 shows it was not until 1938 that the macromolecular nature of DNA was demonstrated by Caspersson and the Hammarstens⁹. Levene then obtained crude extracts of the depolymerising enzyme, DNase, and confirmed the Swedes' work. Did he then denounce the tetranucleotide hypothesis?—No! In 1931 he had conceded the possibility that DNA might be a multiple of the tetranucleotide, and in 1938 he was still considering the possibility of depolymerising the macromolecule right down to "a single tetranucleotide"^{10,11}. Nor was he alone in this. The great German nucleic acid chemist, Robert Feulgen, was also convinced that depolymerisation yielded a product with the tetranucleotide structure¹². Even Gulland accepted the polytetranucleotide as a working hypothesis in 1944, but thought better of it a year later and introduced the 'statistical tetranucleotide' to describe a polynucleotide with base composition equal to that for a tetranucleotide, but with a 'random' sequence^{13,14}.

The clearest evidence for the tetranucleotide, it had seemed, was the supposed existence of one secondary phosphoryl group for every four primary groups (Fig. 1). Only by assuming a four-membered chain could the ratio of 1:4 be explained. If DNA was a very long chain this old titration data of Levene's school must have been wrong, or the DNA studied must have been degraded. Gulland and his colleagues at Nottingham repeated these electrometric titrations and found an "almost entire lack of secondary phosphoryl dissociation"¹⁵. This work was published in a symposium of the Society for Experimental Biology in 1947. It was widely read with the result that attempts were made to improve extractive procedures and prevent the action of DNase. This in turn yielded less degraded DNA from which it was possible to obtain good X-ray diffraction patterns.

Bacterial transformation by DNA

The overriding claim of the proteins to be the hereditary substance rested on the demonstration of their biological specificity in enzymes, antigens, hormones, and viruses. The discovery of nucleic acid in the latter did not alter the situation, since no differences between the nucleic acids of different virus strains could be detected. Therefore, the claim of Avery, MacLeod and McCarty in 1944¹⁶ that DNA can cause a specific heritable alteration to a bacterium found no support from experiments in any other quarter. Nevertheless, this work was not neglected, unknown, undiscussed. On the contrary it sparked off several important researches. These led to the discovery of base ratios and the Boivin—Vendrelly rule.

The first to claim that bacterial transformation was achieved by DNA in another bacterium was the French scientist, André Boivin. Although subsequent attempts to repeat his work failed for many years (until 1972), many scientists were impressed by his work. It led him to seek for features in the metabolism of DNA that one would expect of the hereditary material—that germ cells should have half as much DNA as the body cells since their chromosome number is halved. In 1948 Boivin, with his colleagues, the Vendrellys, was able to announce a halving from 6.5×10^{-6} daltons to 3.4×10^{-6}

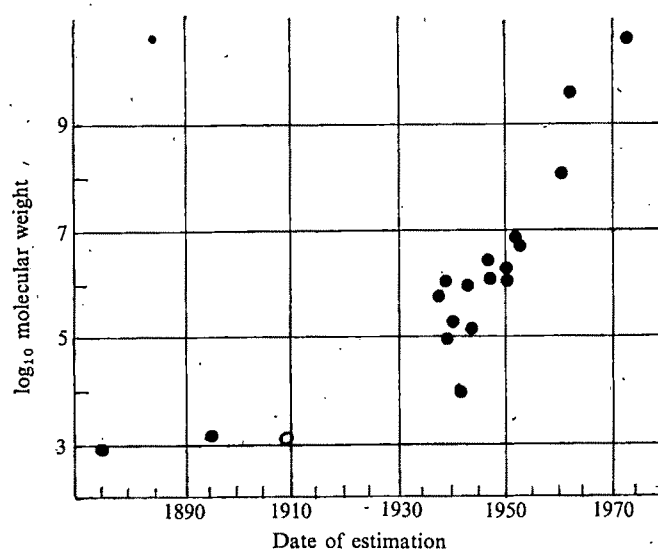


FIG. 2 Molecular weight estimates of DNA. The highest value comes from *Drosophila* DNA; see *Chromosoma*, 48, 1 (1973).

daltons (ref. 17). The significance of this halving rule (the Boivin-Vendrelly rule) was brought out by parallel studies of the metabolic turnover of DNA.

Since 1943 the studies of radioactive incorporation of ^{32}P by Hevesy and his colleagues in Sweden had made plain the contrast between DNA and other cellular compounds, including RNA¹⁸. Its turnover was but a fraction of the others. Also N. J. Davidson, at St. Thomas' Hospital, London, tried starving rats of protein for two days and observing the effect on the DNA and RNA levels in the liver. Whilst the former was unaffected, the latter dipped¹⁹. Boivin took up Davidson's idea and in 1948 a group in Paris presented more striking results which they claimed justified the following conclusions:

"... the absolute quantity of desoxyribonucleic acid enclosed in each nucleus maintains an unchanging value in the course of the most severe fast ...

The invariability of DNA appears as a natural consequence of the special function which is now attributed to it, that of being the depository of the hereditary characters of the species. As for the variability of the RNA; this is easily explained by the very active role which one is inclined to give it in the process of cellular synthesis" (Brachet and Caspersson)²⁰.

At a time when isotopic studies were revealing the very rapid turnover of metabolites here was DNA maintaining a much slower turnover. When Mirsky and Ris confirmed the Boivin-Vendrelly rule they also showed how very variable were the quantities of their 'residual protein'—8.5% to 29% of the chromosomal mass, whereas DNA only varied between 26% and 39%. Now it became clear that the earlier reports of wide fluctuations in DNA percentage content were artefacts. They reflected changes in the absolute quantities of chromosomal proteins. Of these, histones were the predominant class, and recent work had shown that their composition in different tissues of the same organism varied immensely, the arginine from 19% in red blood corpuscles to 87% in mature sperm. Mazia pointed out that in the sperm cell of fish the histone type protein has deteriorated into a much simpler protamine type.

"The sperm cell has no function other than to serve as a genetic bridge between two generations; its physiological capacities are limited to activities that will facilitate the transmission of a nucleus into the egg. If the basic protein fraction actually degenerates in the one case

where we can prove that a full genetic complement is transmitted by the nucleus, it is not tempting to think of this fraction as embodying the properties of genes"²¹.

As far as the apparent metabolic inactivity of DNA was concerned, Mazia saw this as agreeing with the 'template' conception of the gene. "The logical implication is that the gene need not 'do' anything but that it merely provides a blueprint for syntheses". In conclusion, he named DNA as the most likely candidate for the role of genetic material.

Mazia was not the only cell chemist to be impressed by the work of Avery, Boivin and Vendrelly. Arthur Pollister at Columbia University, Hewson Swift at Chicago and Max Alfert at Berkeley were all stimulated by these pioneers to establish further parallels between the genes and DNA, and when a discussion meeting was held at Oak Ridge in 1950 on current problems in the biochemistry of nucleic acids, Pollister spoke warmly of Boivin and described the work of the Vendrellys. Nor was Sir Cyril Hinshelwood, the student of bacterial metabolism, unaware of the Boivin-Vendrelly rule, and from his laboratory of physical chemistry in Oxford came evidence for the constancy of the DNA content and the variability of the RNA content of bacterial cells grown under a wide range of conditions. After suggesting a nucleic acid code for amino acid sequencing (but not a one-way code) Caldwell and he concluded:

"The relative stability of the desoxy-component, and its constancy per cell, perhaps reflect its biological function, in so far as it is believed to be the principal seat of the unchanging hereditary characters, in contrast with the more changeable ribose nucleic acid component. This appears in part to be responsible for the bulk of the protein synthesis, and will presumably be the seat of the variable and adaptable characters"²².

Like Boivin, Erwin Chargaff, at the College of Physicians and Surgeons, New York, was impressed by Avery's work and decided to look for chemical differences in the base composition of DNAs from different sources. In so doing he overthrew the tetranucleotide hypothesis and provided Watson and Crick with the most significant clue to their model—the 1:1 base ratios. It is noteworthy that this achievement was only made possible by the development of chromatography. Just as nucleic acid research benefited from new techniques of molecular weight determination after they had been first applied to the proteins, so it now benefited from the introduction into protein chemistry of paper chromatography. Working under Chargaff, the Swiss chemist, Ernst Vischer, adapted the technique to nucleic acid decomposition products. Fortunately they worked on strikingly different DNA types—the tubercle bacillus (with high content of guanine and cytosine) and yeast DNA (with high content of adenine and thymine). Despite this contrast there was a remarkable approach to equality in the ratios A/T and G/C as in Table 2. In 1950 *Experientia* carried Chargaff's paper on "Chemical Specificity of Nucleic Acids. . ." In his conclusion he affirmed the existence of "an enormous number of structurally different nucleic acids: a number, certainly much larger than the analytical methods available to us at present can reveal"²³. He went on to calculate the number of possible sequences exhibiting the same base proportions as those of the ox. For a chain of 100 nucleotides it was

TABLE 2 Base ratios obtained by Vischer, Zamenhof and Chargaff in 1949

DNA source	Adenine	Thymine	Guanine	Cytosine
Calf thymus	1.7	1.6	1.2	1.0
Beef spleen	1.6	1.5	1.3	1.0
Yeast	1.8	1.9	1.0	1.0
Tubercle bacillus	1.1	1.0	2.6	2.4

10^{50} , for 2500 the number was 10^{1500} . When he addressed a symposium on cytochemistry in 1950 he started by referring to Schrödinger's concept of the hereditary codescript and he went on to give evidence in support of the association of DNA with it. He drew attention to the extension of Avery's work to *E. coli* and *Haemophilus influenzae*. He described his own results, including the classification of DNA into AT and GC types. These facts show that Avery's work did provide the direct stimulus for the most important development in the chemistry of the nucleic acids of that time. No one could claim that Chargaff failed to emphasize the importance of his work, but he failed to achieve a structural explanation of the base ratios.

DNA comes out on top

By the time Watson and Crick came to study the structure of DNA a revolution in the biochemistry of nucleic acids had swept away the old idea of a nucleic acid cycle; a new technique in analytical chemistry had broken the back of the tetranucleotide hypothesis; and from physical chemistry had come new techniques which spelt out the polymeric nature of DNA. By 1950 the chemical and biological specificity of DNA was emerging. To be sure there were qualifications and doubts, halfway positions—the gene is DNA and protein—and just sheer ignorance of these developments. They took several years to spread, and would have taken longer had not the Watson—Crick model speeded up the process. In the meantime one had to read the right literature or belong to the right magic circle. Watson and Crick, by their own admission, did not at first read the literature of nucleic acid chemistry. Most of their clues came from the Phage Group, which until 1952 was ambivalent over DNA and protein. On the other hand members of the Phage Group were not bothered with chromosin or chromosomin; they knew that the nucleic acid of the phage entered the host cell, and by 1952 they were very doubtful that any protein did. On the other hand they were rather arrogant about chemists, so it was not from the Phage Group that Watson and Crick learned about the base ratios, but from Chargaff

himself, when they met him in Cambridge in 1952. Some nine months after that meeting they published their famous model in *Nature*. The key to the model was the pairing of the bases, and this was done in accordance with the Chargaff rules—adenine with thymine and guanine with cytosine, so that $A/T = G/C = 1$. Now, 21 years later, what a rich harvest has been reaped from that neat idea!

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New directions in molecular biology

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"Much has been written about the philosophical consequences of molecular biology. I think it is now clear what the entire enterprise is about. We are looking at a rather special part of the physical universe which contains special mechanisms none of which conflict at all with the laws of physics."

MOLECULAR biology is nothing more than the search for explanations of the behaviour of living things in terms of the molecules that compose them. It is therefore quite an old subject, older than the discovery of the structure of DNA which Watson and Crick announced in this journal 21 years ago. Biochemistry existed as a major scientific enterprise for many years before 1953 and it had made considerable advances in our understanding of intermediary metabolism and biosynthesis in molecular terms. When the double helix appeared it made very little immediate impact on the biochemical establishment which was largely preoccupied with questions about the transformation of matter and energy in

biological systems. It was also viewed with suspicion by most biologists who felt that the complex phenomena of heredity and development could not possibly be explained by what appeared to them to be an excessively simplistic theory. The strong response to the discovery came from quite a small group who took up the new ideas with great fervour. My memory of the early days is that of a small and rather select evangelical movement which often experienced great difficulties in convincing the disbelieving heathen. Looking back, one can now see that the double helix brought the realisation that information in biological systems could be studied much in the same way as energy and matter. It turned genes into chemical objects whose structure and function could be analysed and understood in terms of biochemical machinery.

How this programme, which was only implicit in 1953, has come to be practically realised is now a matter of history. Everybody knows that it depended on the development of powerful methods for fractionating and detecting large molecules, on advances in physical instrumentation and on the judicious choice of organisms for research and their ingenious

genetic manipulation. There were also two other factors which conditioned the explosive growth of the subject and which are not generally emphasised. The double helix fundamentally changed the image of biology. To most young people of my generation the biology taught in universities was a most unattractive subject. It seemed to consist in learning long dusty lists of Latin names punctuated by cutting up frogs or carrots in long dusty laboratories. DNA changed all of that and turned biology into an exciting, intellectually attractive, subject. As a result, large numbers of extremely talented and clever young men who might otherwise have become physicists were drawn to it and the edifice of molecular biology that we have today owes much to their ingenuity and hard work. The second factor was simply the massive expansion of governmental financial support for science and higher education, particularly in the United States, which provided the material base for the rapid growth of molecular biology. Watson and Crick may have invented it but Uncle Sam certainly fuelled it.

Communication

So much for the past; this article is supposed to be concerned with the future. To try to pick out new directions in molecular biology and to do it in a way acceptable to most people has proved to be an impossible task. One of the consequences of the growth of the subject is the vast literature it has generated which nobody can read in its entirety. I estimate that the leading journals alone produce about 10^5 pages of material per year which, in all scholarly conscience, ought to be studied. Nobody can do this and most molecular biologists tend to be conversant with only a small part of the field and rely on others telling them what is interesting or new elsewhere. The rumour, propagated by word of mouth or telephone, has become the main vehicle of communication. If you want to keep something secret in molecular biology, you publish it but do not talk about it. New directions and new ideas in the subject are often generated by one or a small number of people but they become rapidly communalised by this anonymous process of verbal transmission. Here they are held in a reverberating form until someone does the crucial experiment. When that happens, growth can be explosive; if nothing happens, the idea simply peters out. It is this concentration on the experiment that gives molecular biology its rather distinctive and ruthless character and it probably explains why (with the possible exception of Francis Crick) there are no living theoretical biologists. To write about new trends in molecular biology is to tackle a very tenuous field and I can only do it in a very general way.

Developmental biology

First it is clear that the new parts of molecular biology are just the old parts of biology. There has been an increasingly popular move from the study of prokaryotic microorganisms to work on multicellular eukaryotes. Those problems of higher organisms that are directly amenable to the methods of molecular biology have fallen rapidly beneath the axe; antibodies and hormone and drug receptors are recent examples and the analysis of intracellular organelles such as mitochondria is another noteworthy instance. The problem that has only barely been dented is that of development. It is a very old problem indeed. At the turn of the century Thomas Hunt Morgan left embryology to enter the new field of genetics in the hope that it might cast light on the intractable problems of development. One view of modern molecular biology is that it has completed Morgan's deviation and we are now back with the same unsolved problems. How do the genes specify the complex cellular structures that we find in higher organisms? What controls the temporal sequence of development, the geographical layout of cells and their connections, and specific biochemical differentiation? It is not good enough to answer these questions by saying it is simply a matter of turning some genes on and others

off at the right times. It is true that molecular biology provides numerous detailed precedents for mechanisms by which this can, in principle, be done, but we demand something more than these absolutely true, absolutely vacuous statements.

It is interesting to ask whether the problems of developmental biology could be solved by one insight as penetrating as that of the double helix. This is really asking whether higher organisms have some unique piece of molecular biology that is unknown to us. There is a strong tendency of molecular biologists to use every new molecular device uncovered as a basis for a new theory of development. Antibody molecules are composed of heavy and light polypeptide chains which are themselves specified by stitching together a variable and a common gene sequence. There have been numerous speculations on applying this mechanism to the generation of molecular diversity in developing systems. My impression is that these ideas exist in a kind of limbo, neither accepted nor rejected, and probably not even generally discussed. The attitude is that there is nothing to talk about until someone either proves that this is a more general mechanism and not something specific to the immune system.

The general belief is that developmental biology will not be solved at one stroke but its problems will be partitioned into subproblems each of which could be tackled separately, preferably in a model system. One example is cell movement and growth. Clearly, how cells move in relation to each other and how they send out processes is central to understanding development. It is now well established that the motility in cells is generated by actin and myosin molecules closely related in structure to their counterparts in muscle. This turns out to be true of amoebae as well so that the same phenomena with the same underlying molecular mechanism can be studied in detail in a simpler experimental system. It is likely that many more analogous cases will be found and that the cytoplasmic mechanisms of higher cells which provide the machinery of development will, one by one, be isolated and studied in this way.

Eukaryote chromosome structure

There are two problems which are at a different stage of study and which are of great interest at the present moment. The first is the structure of the chromosomes of eukaryotes; the second, the cell surface. It is by now well known that the DNA of higher organisms presents a strange paradox. In brief, there seems to be too much of it for what we think it ought to be doing. When genetic results are compared with the unique sequence content of the DNA in different organisms, the genetic units turn out to be very large. In *Drosophila*, an average gene contains about 20,000 base pairs which is much larger than the 1,000 base pairs required to code for the average polypeptide chain. This is over and above the problem posed by the presence of an even larger excess of redundant sequences. The chromosomes of higher organisms also differ from those of prokaryotes by their content of specific proteins like histones and their far more complex structure. Many theories have been proposed to explain these observations, in terms of the special structural or control requirements of the cells of higher organisms. Solving the structure of chromatin is a very active field at the present moment and success is likely to throw much light on these problems.

An interesting consequence of this new work is that the number of genes in higher organisms is much smaller than we might have been led to expect from their DNA composition. It suggests that *Drosophila* might have as few as 5,000 genes and the small nematode *C. elegans* perhaps only half that number. Using various methods of maintaining lethals it should be possible to isolate mutants in every gene in such organisms. For each of these mutants, as has already been done in a few instances, one could look at the developmental consequences and, with temperature-sensitive alleles, one

could determine the time of action of the gene. Could we gain a detailed understanding of development from this approach? One view is that this might allow us to deduce the logical structure of the genetic programme by seeing whether there are partitioned subsets of genes which correspond in some interesting way to the phenotype. Questions of this sort are not strictly molecular being concerned more with the software of organisms than with their hardware. In the long run, however, we must find the molecular implementations. This would involve identifying the products of the genes and finding out which cells have them and where and when they work to produce their effects. But to do this requires high resolution protein spectroscopy and this technology only exists in a crude and cumbersome form today. Not many people would be willing to embark on such a programme because of its intrinsic magnitude, and there are also many workers who would object on grounds of principle to this approach. There is in fact a fairly large antigenetic school of molecular biologists who believe that the work should proceed from the biochemistry and not the other way around. The only difficulty is that, except in special cases, nobody knows what biochemistry ought to be done.

Cell surface

This brings me finally to the question of the cell surface. One of those nebulous communally circulating ideas is that the cell surface contains the key to the mechanisms of development. These notions have been generated from many different sources. Some come by extrapolation from cell interactions in the immune system, others from the develop-

ment or regeneration of neural connections, and yet others from the study of the reaggregation properties of embryonic cells. The central thought is that cells or groups of cells carry a specific chemical code on their surfaces and by this code they know themselves and their neighbours. In the pages of this journal there have appeared various baroque forms of this theory in which gene stitching, reverse transcriptase and other pieces of molecular clockwork have been grafted onto the idea of specific chemical labelling of cells. Nobody will deny that cells do have surface proteins and that some of these will be involved in recognising specific chemical signals such as nerve transmitters or hormones. The real question is whether there really exists a system which, by one mechanism or another, can generate 10^6 chemical names and plant these on the right cells. There seems to be no convincing general argument either for or against this view and only time will tell.

Much has been written about the philosophical consequences of molecular biology. I think it is now quite clear what the enterprise is about. We are looking at a rather special part of the physical universe which contains special mechanisms none of which conflict at all with the laws of physics. That there would be new laws of Nature to be found in biological systems was a misjudged view and that hope or fear has just vanished. Our job is simply to find out how these interesting pieces of machinery work, how they get built and how they came to be the way they are. In one sense, the answers already exist and all we have to do is to find out how to look them up in Nature. That is why molecular biology seems to me to be the art of the inevitable.

Rosalind Franklin and the double helix

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A draft manuscript shows how near Rosalind Franklin came to finding the correct structure of DNA.

SOME years ago I gave an account¹ of Rosalind Franklin's contribution to the discovery of the structure of DNA, based on published sources supplemented by references to her notebooks and reports. I pointed out how close Franklin had come in the progress of her work to various features of the structure contained in the correct solution. At the time, however, I did not know of the existence of a draft manuscript which confirms how close she had got to the answer. This only came to light later. I therefore take the opportunity of this 21st Anniversary issue to fill out the record and to highlight a dramatic element in the 'race' for DNA.

In my article I told how the analysis of the B form of DNA in terms of helical diffraction theory, which is given in Franklin's paper with Gosling in *Nature*² on April 25, 1953, can be found in her notebook for the period January to March 1953, that is before the Watson-Crick structure had become known to her. I went on to say, however, that she apparently did not feel convinced enough of the relevance of this analysis to publish it (because she had not solved the A form). This is erroneous. The typescript I have found is dated March 17, 1953 and is clearly a draft of the *Nature* paper. This suggests a different explanation from the one I gave, namely that she was proposing to publish what she knew on the B form, two other papers on the A form having already been submitted (before March 6) to *Acta Crystallographica*. This draft contains all the essentials of the *Nature* paper, and much of the wording is carried over intact. It required only slight modifications to take into account the Watson-Crick structure, news of which reached

King's College on March 18, one day later.⁴ In the final (undated) typescript, there is inserted by hand the sentence "Thus our general ideas are not inconsistent with the model proposed by Watson and Crick in the preceding communication."

In the draft it is deduced that the phosphate groups lie on a helix of diameter 20 Å, that is, on the outside of the molecule, in accordance with the conclusion Franklin had reached earlier on the basis of physicochemical reasoning, including her own work on the water uptake by DNA fibres undergoing the A-B transition. Moreover, on the basis of the intensity distribution in the X-ray pattern, she concludes that there is not one chain in the helix, but probably two, coaxially arranged, and that these are separated by $\frac{3}{8}$ of the period, or 13 Å, along the fibre axis direction. The wording is so couched, however, as to show Franklin had not yet understood that the two chains must run in opposite directions, although she had already observed in her notebook that this must be the case in the crystalline A form (which has a two-fold axis of symmetry). I have already argued that it would not have taken long for her to see the true relationship between the two forms—at the time she was thinking of the A form as an unwound version of the helices in the B state (rather, I imagine, like the β -sheet structure is to the α helix in polypeptides).

This would have brought her to the final and crucial step necessary to the complete solution, base pairing. Franklin had thought from an early stage that the bases must lie on the inside of the molecule and be linked by hydrogen bonds, and she had already formed the notion that the two purines were interchangeable with each other and also the two pyrimidines³. An entry in her notebook shows that she was considering Chargaff's analytical data, though there is nothing

to show that she knew the correct tautomeric forms of the bases. The step from base interchangeability to base pairing is a large one, but the idea would have been essential to fitting the variable parts of the structure, the bases, in, to the regularly repeating part, the double helix of phosphate-sugar chains at which she had arrived by March 1953.

¹ Klug, A., *Nature*, 219, 808-810, 843-844; also 880 (1968).

² Franklin, R. E., and Gosling, R. G., *Nature*, 171, 740-741 (1953).

³ Franklin, R. E., and Gosling, R. G., *Acta Crystallogr.*, 6, 673-677 (1953).

⁴ Olby, R. C., *The Path to the Double Helix* (Macmillan, London, in the press).

Molecular biologists come of age in Aries

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It seems that more molecular biologists are born under Aries than any other sign.

ONE major tenet of astrology is that the 'Sun sign' (the constellation of the zodiac in which the Sun was located at the moment of a person's birth) has a powerful influence on

personality¹. I have compared the birthdays of two very different kinds of biologists—taxonomists and molecular biologists. All names listed under all of the taxonomy terms (cyto-, insect, and plant taxonomy; systematic botany, entomology, ichthyology, and zoology; systematics; taxonomic botany, and taxonomy) and all of the molecular biology terms (molecular biology, biophysics, genetics, pharmacology)

TABLE 1 Relative frequencies of births under the different Sun signs

Sun sign	Taxonomists			Molecular Biologists			General Population		
	No.	%	Index	No.	%	Index	Month	%	Index
Aries	28	8.2	98.2	58	12.3	148.0	March	8.4	101.4
Taurus	30	8.8	105.3	32	6.8	81.6	April	8.1	97.1
Gemini	31	9.1	108.8	39	8.3	99.5	May	8.1	97.5
Cancer	38	11.1	133.3	41	8.7	104.6	June	8.2	98.9
Leo	32	9.4	112.3	32	6.8	81.6	July	8.6	103.7
Virgo	31	9.1	108.8	42	8.9	107.1	Aug	8.7	104.8
Libra	25	7.3	87.7	41	8.7	104.6	Sept	8.8	105.4
Scorpio	18	5.3	63.2	41	8.7	104.6	Oct	8.2	98.4
Sagittarius	27	7.9	94.7	40	8.5	102.0	Nov	8.0	95.6
Capricorn	25	7.3	87.7	33	7.0	84.2	Dec	7.9	94.6
Aquarius	26	7.6	91.2	35	7.4	89.3	Jan	8.3	100.0
Pisces	31	9.1	108.8	36	7.7	91.8	Feb	8.6	102.7

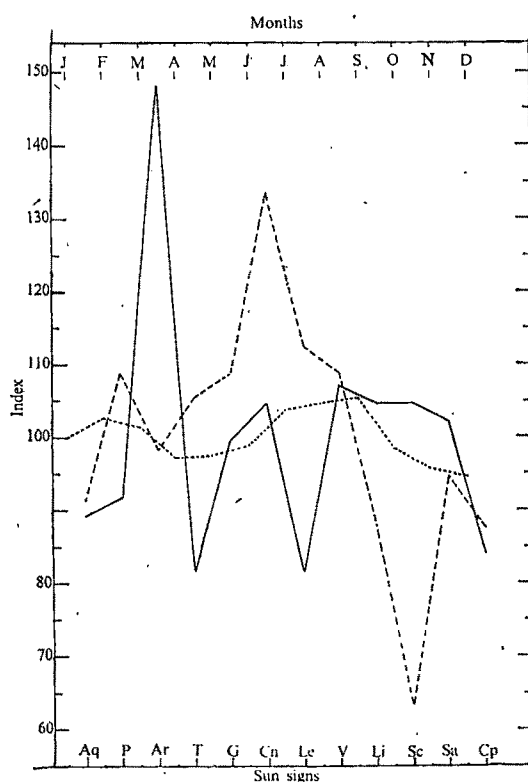


FIG. 1 Relative frequencies of births, expressed as index values, under the different Sun signs for taxonomists (---) and molecular biologists (—); also shown are monthly birth data for the United States in 1934 (....).

were taken from the Discipline Index² of the twelfth edition of *American Men and Women of Science*³. The birthdays were obtained from the main reference portion and the appropriate sun signs were assigned. Names appearing more than once were only counted once. A great deal of variation occurs in the dates for these signs because the sun does not always change signs at midnight, so I used the dates given by Goodman¹. Not all scientists listed had birthdays given. The numbers of persons born under each sign were tallied and the relative frequencies were expressed as an index (each value divided by the average, times 100)⁴ (Table 1, Fig. 1). The monthly birth data for the general population of the United States for 1934 are given for comparison; this is the year closest to the births of most of the scientists now recorded, for which good data are available.

More molecular biologists were born under the sign of Aries than any other sign. More taxonomists were born under the sign of Cancer than any other sign and relatively few were born under Scorpio. Since these peaks do not coincide with the peaks for the general population and are sometimes contrary to them, they are even more remarkable.

¹ Goodman, L., *Linda Goodman's Sun Signs* (Bantam Books, Taplinger Publishing Co., New York, 1968).

² *Discipline Index. American Men and Women of Science. The Physical and Biological Sciences*, twelfth ed. (edit. by Jaques Cattell Press), 135, 231, 304-306, 469, 511-512 (Bowker, New York, 1973).

³ *American Men and Women of Science. The Physical and Biological Sciences*, twelfth ed. (edit. by Jaques Cattell Press) (Bowker, New York; A-C 1:1-1238 (1971), D-G 2:1239-2392 (1972), H-K 3:2393-3496 (1972), L-O 4:3497-4730 (1972), P-Sr 5:4731-6044 (1972), St-Z 6:6045-7184 (1973)).

⁴ Rosenberg, H. M., *Vital Health Statistics*, 21(9), 1-59 (1966).

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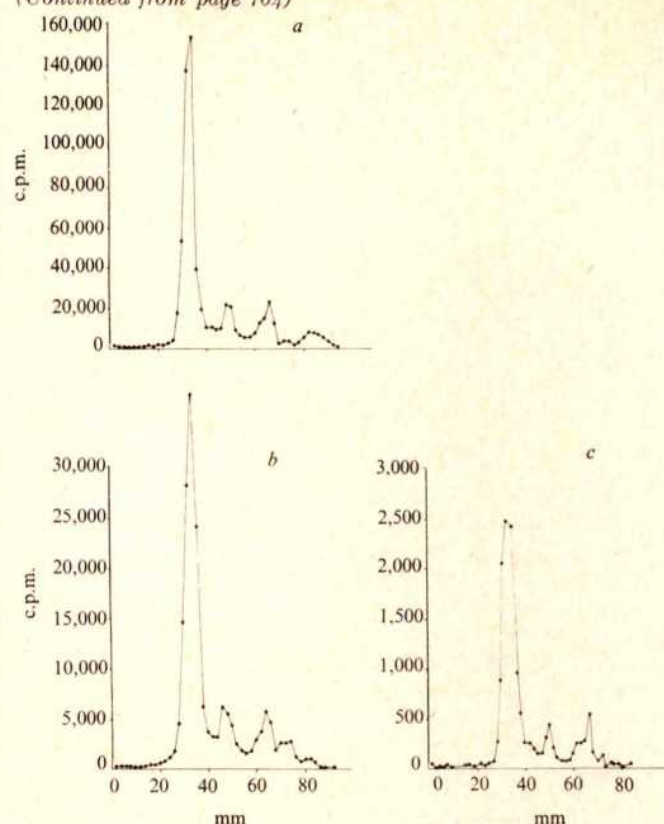


FIG. 2 Separation of iodinated rat erythrocyte polypeptides. Erythrocytes were isolated and iodinated as described in the text. Labelled cells were injected into animals and blood samples were collected at various times thereafter. Erythrocytes were washed free of serum proteins, and the plasma membranes were isolated. Membranes were then solubilised in 2-3% SDS and separated by electrophoresis on 5% polyacrylamide gels containing 0.1% SDS⁶. The distribution of radioactivity in the gels was determined by slicing the gels into 2 mm slices and then determining the radioactivity in each slice. The profiles shown are from erythrocyte membranes isolated from animals 1 h (a); 19 h (b); and 404 h (c), after injecting labelled cells.

components of larger molecular weight are degraded^{5,6} more rapidly. In the erythrocyte membrane this apparently is not the case; all components seem to have approximately the same half-life which is significantly shorter than the half-life of the whole cell.

There may be several reasons for the short half-life of membrane components observed with the iodine probe. One possible reason is that the labelled components are de-iodinated. There is a report of de-iodination activity in the erythrocyte membrane¹⁶. It is also possible that the proteolytic activity^{17,18} present in the red cell may account for the removal or loss of label due to proteolysis of the membrane component.

The most probable explanation for the short half-life observed with the iodine labelling is based on the observation that young erythrocytes are larger than old ones. The decrease in cell size is probably due to sloughing off of the membrane (see, for example, ref. 19) within the sinusoids of the spleen and liver. This process takes with it relatively small quantities of the internal components of the red cell, but probably relatively significant quantities of membranes with much smaller losses of the internal components of the red cell. Thus, by this mechanism, iodine would not be eluted in the same manner as chromium is presumed to be eluted, but would represent loss of membrane from the cell during the course of its life. Thus, membrane labelling may have advantages in the study of membrane diseases and the survival of the membrane in contrast to the interior of the cell. This method may provide information about the activity or

functional capacity of the spleen and hepatic sinusoid cells in diseases where it is possible that these organs and not the erythrocytes are defective.

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Novel human serum protein from fibroblast plasma membrane

CELL surface components play a major role in the control of normal cell interactions and in the altered growth control and antigenic properties of malignant cells¹. To characterise tissue-specific cell surface proteins we undertook an immunochemical study of the material released from cells by mild proteolytic treatment. Our studies have disclosed a fibroblast-specific cell surface and serum antigen in chicken². The amount of this antigen is greatly reduced in chicken fibroblasts transformed by Rous sarcoma virus.

These studies prompted us to search for an analogous protein in humans in light of its possible significance in human malignancies. We describe here a new cell surface protein of human fibroblasts which is also present in human serum, and seems to be the human counterpart of the chicken fibroblast surface antigen.

Antisera were raised against material prepared from the surface of human secondary fibroblasts with insoluble papain² (Enzite-EMA, Miles-Yeda). The antiserum prepared against the papain-released material from fibroblasts, gave a single precipitation line against the immunogen and similar material from four continuous fibroblasts lines: WI-38 (ref. 3), MRC-5 (ref. 4), IHM (human embryonic fibroblast) and 377HEL (human embryonic fibroblast). An antigen in-

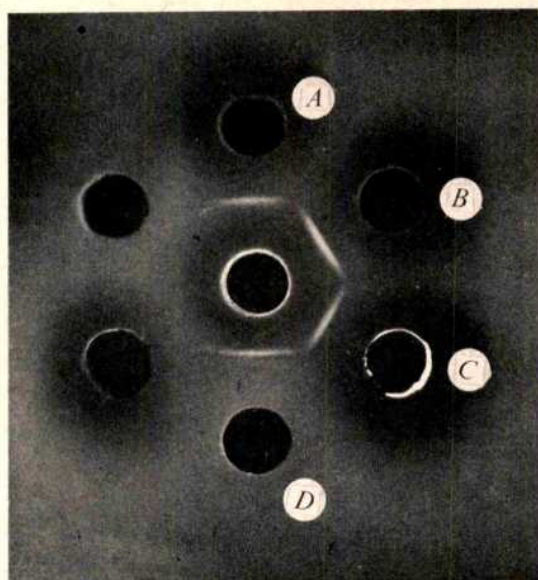


Fig. 1 Immunodiffusion in agarose. A and D, Normal human serum; B, papain digest from MRC-5 cells; C, MRC-5 cells extracted with 8 M urea and 1% Triton X-100. Centre well, rabbit antiserum prepared against papain digest from human fibroblasts.

distinguishable from the fibroblast antigen in immunodiffusion and absorption experiments was present in human serum (Fig. 1). The antigen could be solubilised from fibroblasts by papain, trypsin, or by treatment with a solution containing 8 M Urea, 1% Triton X-100, 5×10^{-5} M phenylmethylsulphonyl fluoride and 0.02% sodium azide. Incubation of the cells in buffered saline or EDTA in conditions comparable to the enzyme treatment did not yield detectable amounts of the antigen. Small quantities could, however, be detected in the maintenance medium of fibroblast cultures after 24 h incubation. The antigen was not detected in material released by papain or urea-Triton X-100 prepared from several non-fibroblast human cell lines. The cell lines included U and WISH transformed amnion lines, primary

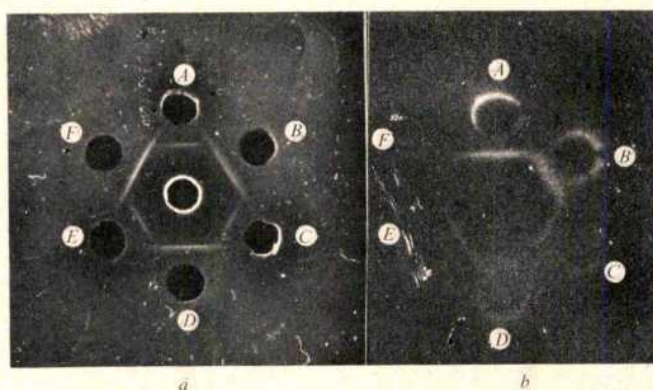


Fig. 2 Immunodiffusion in agarose (a) followed by autoradiography (b). A, C and E, Normal human serum; B, D and F, foetal extract. Centre well, rabbit antiserum prepared against papain digest from human fibroblast and anti- α -foetoprotein serum. Papain digest from H^3 -fucose labelled MRC-5 cells was added to the peripheral wells, undiluted (A and B) diluted 1:4 (C and D) and 1:16 (E and F). The highest amount of H^3 -fucose labelled digest, in well B, gives a precipitate without carrier antigen from normal human serum. Smaller concentrations in wells C and E coprecipitate with the line between normal human serum and antiserum, while the unrelated control precipitate (alpha-foetoprotein) from wells D and F remains non-radioactive.

normal amnion, HeLa cervical carcinoma, KB (nasopharyngeal carcinoma), Hep-2 (laryngeal carcinoma), P3HR-1 (Burkitt's lymphoma). Material released by papain from washed red blood cells and buffy coat leukocytes was also negative.

That the antigen is synthesised by the fibroblasts and not absorbed from serum was shown by its presence in the established human fibroblast lines grown for over 30 passages in the presence of calf serum which does not contain the human antigen. Cells grown in the presence of 35 S-methionine incorporated radioactivity to the antigen as shown by autoradiography of immunodiffusion precipitates. Similarly, radioactivity was incorporated into the antigen from 14 C-fucose (Fig. 2). Iodination (125 I) using the peroxidase method⁵ labelled the antigen as demonstrated by coprecipitation of radioactivity into a specific immunoprecipitate.

Assay of the antigen after solubilisation of cells in urea and Triton X-100 showed that treatment with trypsin (0.25%, 15 min), such as used in the subcultivation of fibroblasts, removed more than 90% of the antigen from cells without any detectable cell destruction. The antigen was fully restored within 24 h after such treatment. Absorption of the antiserum with living fibroblasts abolished its precipitating capacity in immunodiffusion. These data show that the antigen is an integral membrane glycoprotein and is exposed on the cell surface. No immunological cross reactions were demonstrable between the human and chicken² antigens. Antisera against the human material did not react with extracts from chicken cells or *vice versa*.

We have determined some properties of this protein for which we propose the name soluble fibroblast antigen (SF antigen). In sedimentation analysis we used 5–20% sucrose gradients and human IgM (19S), IgG (7.1S), albumin (4.3S), ovalbumin (3.6S) and pancreatic RNase (1.9S) as reference markers. The S values were calculated according to Martin and Ames⁶. The serum SF antigen sedimented during ultracentrifugation in the 13.5S region. In agreement with the relatively high molecular weight suggested by the S value, the serum SF antigen was precipitated by 25% saturated ammonium sulphate. On immunoelectrophoresis, the serum SF antigen gave a precipitate in the α_2 -region (Fig. 3). The presence of sialic acid in the antigen was indicated by an altered electrophoretic mobility after neuraminidase (Behringwerke) treatment of partially purified serum antigen (Fig. 4). Pronase (Calbiochem, 537088) treatment of partially purified SF antigen destroyed its antigenic activity. For inactivation by Pronase it was necessary to use slightly larger amounts than those required to abolish antigenic reactivity of human serum albumin. Human blood group A substance, used as another control, was not affected by the treatment. These data indicate that the polypeptide chain is involved in the antigenic activity.

The serum SF antigen could be demonstrated in all 60 normal human sera studied and also in smaller quantities in 2 foetal sera. We are now investigating the quantity and possible compositional deviation of the serum SF antigen in various forms of connective tissue disease and in malignancies. The absolute concentration of SF antigen in serum or cells cannot be given at present, because attempts to purify the serum SF antigen in immunoreactive form have so far been hampered by its insolubility after purification procedures. This is in line with the postulated membrane origin of the serum SF antigen, as membrane proteins tend to be hydrophobic. The antigen, however, was detectable in immunodiffusion (sensitivity about $10 \mu\text{g ml}^{-1}$)⁷, in normal human serum diluted 1:10 and in fibroblast samples containing 2.0 mg ml^{-1} of total cell protein. This result indicates that the serum concentration of SF antigen is about $100 \mu\text{g ml}^{-1}$ and it may comprise as much as 0.5% of the total cell protein, which would make it a major component of the plasma membrane.

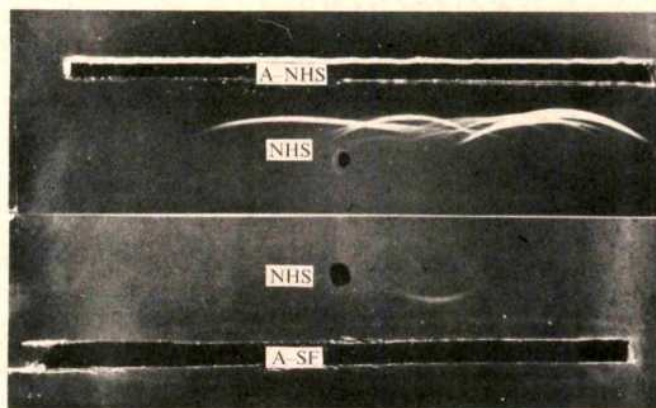


Fig. 3 Immunoelectrophoresis in agarose. Normal human serum (NHS) tested against anti-normal human serum (A-NHS) and an antiserum prepared against papain digest from fibroblasts (A-SF). Anode to the right.

The SF antigen seems to be the first well defined example of a new type of serum protein, which originates from the cell surface. Other cell surface components, also seem to exfoliate from the cell membrane and appear in circulation, albeit at minute concentrations⁸⁻¹⁰. That the SF antigen is present in serum at high concentrations may be because of the abundance of fibroblasts in the body.

Our recent data (P. Kuusela, E. R., and A. V., unpublished) indicate that the polypeptide composition of the SF antigen in serum and in fibroblasts is very similar, consisting of two major polypeptide chains with molecular weights of about 210,000 and 145,000. This is true of human as well as chicken SF antigens. This shows that the similarity of the cellular and serum antigens is not restricted to a limited antigenic determinant, but is based on a molecular similarity if not identity. This, and the fact that cultured fibroblasts shed the antigens to growth medium, strongly supports the notion that the serum antigen originates from the fibroblast surface.

As a well defined glycoprotein SF antigen may offer a unique possibility to look into the biological significance of circulating cell surface components, it has been suggested that growth regulation¹¹ and the maintenance of immunological tolerance¹² could be mediated by such molecules.

It is predictable that cell-type specific surface glycoproteins, such as SF antigen, may be important for the interactions between normal cells and perhaps function as recognition molecules¹³⁻¹⁶. Our observation that reduced amounts of the chicken SF protein², analogous to the human protein described here, are present in virus transformed cells¹⁷, is in line with this proposal. Our recent data (A. V., and E. R.,

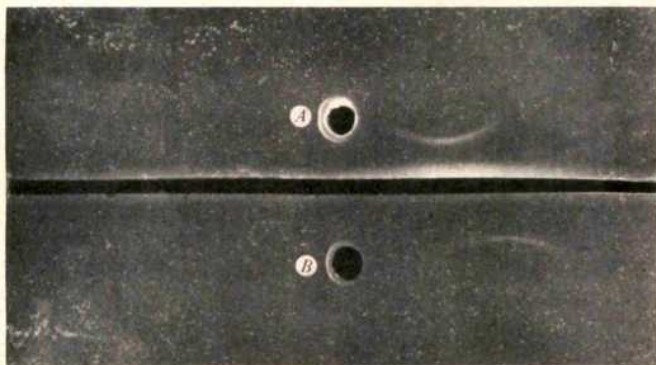


Fig. 4 Immunoelectrophoresis in agarose. Effect of neuraminidase on the electrophoretic mobility of partially purified SF antigen. A, Neuraminidase treated; B, control treated (without neuraminidase) SF antigen tested against A-SF serum.

unpublished) indicate that human fibroblasts also lose their SF antigen after transformation. This indicates that the biological role and the possible diagnostic significance of the human SF antigen and related cell surface components in human malignancies deserve further attention.

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Composition drift in the cytochrome c cistron

In a previous letter I showed that the distribution of base frequencies for degenerate codon positions is far from random in the only completely sequenced mRNA, the coat cistron of MS2 coliphage¹. The study reported here indicates a strong evolutionary trend among non-degenerate bases in mRNA for the cytochrome c series leading to man.

Forty-three best resolved cytochrome protein sequences have been decoded to obtain theoretical mRNA base compositions, assuming equal use within the set of codons of each amino acid¹. The adenine content in each theoretical mRNA is correlated with the estimated palaeontological age for that type of organism (Table 1). Although fewer fish and insect sequences are available, each age is fairly well represented. Many cytochrome c molecules from plants have been sequenced but only one, ginkgo, which is believed to be the most primitive² and thus the closest to the line leading to man, is included to avoid weighting plants too heavily in the correlation. Simply decoding every cytochrome c ever sequenced, however, while making a bulkier analysis, would not greatly affect the trends shown.

TABLE 1 Adenine content in cytochrome mRNA

(a) Palaeontological age and adenine content of theoretical mRNA									
Bacteria, alga ^{4,11,14-17} (3 × 10 ⁹ yr)	%A	First eukaryotes ^{2,4,18-21} (1.5 × 10 ⁹ yr)	%A	Insects ⁴ (800 × 10 ⁶ yr)	%A	Reptiles, birds ^{4,23} (300 × 10 ⁶ yr)	%A	Mammals ^{4,24} (80 × 10 ⁶ yr)	%A
<i>Pseudomonas fluorescens</i> c551	28.7	<i>Crithidia oncopelti</i>	28.9	Tobacco moth	31.5	Pekin duck	35.7	Bat	35.1
<i>P. aeruginosa</i> c551	27.8	<i>Euglena gracilis</i>	31.3	Silkworm moth	31.7	Rattlesnake	35.8	Dog	35.8
<i>P. stutzeri</i> c551	27.2	Ginkgo	31.6	Screw-worm fly	31.7	Chicken, turkey	36.0	Whale	36.0
<i>P. mendocina</i> c551	25.7	<i>Candida krusei</i>	30.0	Fruit fly	31.8	King penguin	36.3	Rabbit	36.1
<i>Monochrysis lutheri</i> c553	27.8	<i>Ustilago sphaerogena</i>	32.5			Emu	36.4	Pig, cow, sheep	36.4
<i>Desulfovibrio vulgaris</i> c553	31.0	<i>Neurospora crassa</i>	32.6	Fish ^{4,22} (400 × 10 ⁶ yr)		Snapping turtle	36.7	Horse, donkey	37.7
<i>D. vulgaris</i> c3	32.9	<i>Debaromyces klockeri</i>	33.0	Carp	32.8				
<i>D. gigas</i> c3	31.8	Baker's yeast	34.2	Tuna	34.0	Bullfrog (350 × 10 ⁶ yr)	35.1	Kangaroo (100 × 10 ⁶ yr)	36.7
<i>D. desulfuricans</i> c3	29.8			Bonito	34.1				
<i>Rhodospirillum rubrum</i> c2	33.4			Puget Sound dogfish	34.7			Man, monkeys (present)	36.6
<i>Chloropseudomonas ethylica</i> c7	34.7			Pacific lamprey (450 × 10 ⁶ yr)	34.8				
(b) Correlation between age and %A in theoretical mRNA									
No. organisms	Span: from man to	-r*	-b*	a*					
24	Insects	0.89	6.5	37.1					
32	First eukaryotes	0.82	3.3	36.2					
43	All bacteria + alga	0.77	2.0	35.6					
36	<i>Pseudomonas</i> c551 only	0.91	3.0	36.1					

* r is linear regression correlation coefficient, b is slope (%A per 10^9 yr), a is intercept at time zero (present).

I have tried to take a least controversial figure for each evolutionary age. Ages of bacteria and algae are generally estimated at around 3×10^9 yr^{3,4}, first eukaryotes at 1.2×10^9 to 1.8×10^9 yr^{5,6} and insects at 600–1,000 Myr^{4,7,8}; agreement is better on dates for more recent organisms (from lamprey onwards). The conclusions of this paper would not change if some of the ages in Table 1 are wrong by 25%–30%.

The adenine content in cytochrome *c* theoretical mRNA has increased by about 5% (absolute) since the appearance of eukaryotes. This is an enormous value in a cistron which already contained a considerable amount of A (29%–33%) at that time and which evolves slowly. Just when the trend started is uncertain, however, because the ancestry of cytochrome *c* in bacteria and algae is not clear^{4,6,9-11,12}. The trend may be accelerating, as a comparison of the magnitudes of the three slopes suggests (human–insect > human–first eukaryotes > human–bacteria and alga, see Table 1b). But perhaps insect divergence goes back almost to the appearance of eukaryotes.

A bacterial or algal ancestor cistron containing 35%–37% A is unlikely because of the lower percentage of A for intermediate organisms (protozoa, insects and fish) accepted as having cytochrome *c* homologous with that of man. Also, all fifteen theoretical mRNAs from bullfrog onwards, on the evolutionary scale, have at least 35% A whereas none of the eleven bacterial and algal ones have as much as 35%. In fact, judging composition trends, *Pseudomonas* c551 is a more likely candidate for the ancestor of mammalian cytochrome *c* than is *Rh. rubrum* c2. Mammalian cytochrome *c* has 72% (relative) more Lys and 38% more of the combined five amino acids besides Lys with a high percentage of A in their codons (Asn, Gln, Glu, Ile, Thr) than has *Pseudomonas* c551. Lysine codons have an average A content of 83.3% whereas codons from the other five amino acids average 50% A. As a generalisation, evolutionary change of each residue in cytochrome *c* is proportional to the adenine content of its codons. This cannot be explained by increasing polarity because two (Ile, Thr) of the five amino acids have low polarities. Furthermore, highly polar Asp (whose codons have an average of 33.3% A) has decreased in frequency while less polar Asn¹³ (whose codons have an average of 66.7% A) has increased during this interval. The evolu-

tionary progression of the six amino acids with codons of high A content is summarised in Table 2.

Neither a classical selectionist nor a neutralist interpretation of these findings seems possible. Unless a bias in the choice of organisms to sequence somehow exists, however, there is no escaping the fact of a general, progressive increase of all codons of high A content in the cytochrome *c* line. There are two conceivable models for the trend. In the first, cistron evolution proceeds by a general increase of codons containing a predominance of adenine while no special control is exerted on degenerate bases. Alternatively, the observed increase in non-degenerate A is compensated by appropriate choices among degenerate bases to contain the drift in total cistron composition¹.

mRNA sequences will help to decide between these models. The actual mRNA in cytochrome *c* will have had to gain considerably in A content unless numerous silent mutations (ones not affecting amino acids) have occurred. This is readily seen by aligning, for example, horse and silkworm moth cytochrome *c* sequences. Ignoring non-homologous ends (last horse and first four moth residues) and noting possible base changes in codons of exchanging amino acids, the adenine content of horse mRNA can be lowered at most by 1% by avoiding A as a degenerate base in replacing codons, whereas the theoretical adenine difference between the two species is 5%.

Other proteins show trends in the choice of non-degenerate

TABLE 2 Evolutionary progression of amino acids with codons of high adenine content

No. of sequences	Type	Average in cytochrome % Lys	% NQEIT*
11	All bacteria and alga ^{4,11,14-17}	13.0	19.4
(1)	<i>Rhodospirillum rubrum</i> c2 ⁴	15.2	24.0
(4)	<i>Pseudomonas</i> c551 ^{4,11}	10.1	21.1
8	First eukaryotes ^{2,4,18-21}	12.9	25.5
4	Insects ⁴	13.6	24.3
5	Fish + lamprey ^{4,22}	16.0	26.0
7	Reptiles, bird + frog ^{4,23}	17.3	28.0
8	Mammals + kangaroo ^{4,24}	17.4	29.2

* Asn + Gln + Glu + Ile + Thr.

codon bases (for example, the theoretical mRNA in haemoglobin α chain has increased sharply in C content during its evolutionary life) but fewer sequences or confirmed compositions are available for each series.

It seems, therefore at least for composition trends, that gene evolution is not following a random course. This seems a genuine case of long-term oriented molecular evolution. Whether or not archaic morphology means archaic genes, the correlation in Table 1 remains to be explained. The question is how independent are genetic and phenotypic evolution (further work in preparation).

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Fusion of fungal protoplasts

RESEARCH on fusion of cells of higher eukaryotes is in an advanced stage and excellent reviews have been published concerning fusion of both animal cells¹⁻⁴ and plant protoplasts⁵⁻⁷. On the other hand, no information is available regarding the results of fusion of fungal protoplasts, in spite of its possibilities for basic and applied research. Here we present evidence that fungal protoplast fusion and heterokaryon formation can be achieved. In our experiments no stimulants of protoplast fusion were employed. In fact, a fungus was used in which we have never observed anastomosis and heterokaryosis or sexual processes.

Nutritionally deficient stable mutants were produced from *Geotrichum candidum* SzMC 0055 with N-methyl-N'-nitro-N-nitrosoguanidine ($50 \mu\text{g ml}^{-1}$, 60 min, 10^4 arthrospores ml^{-1}). Auxotrophic mutants requiring adenine (*ad*), lysine (*lys*), isoleucine, and methionine were used. Nutritional complementation by protoplast fusion could be achieved with any pair of these auxotrophic mutants, but results reported here refer to the *ad* and *lys* mutants.

Protoplasts were formed from rapidly growing hyphae ($100 \text{ mg fresh weight ml}^{-1}$) after 2-mercaptoethanol treatment⁸ by using lyophilised snail digestive juice (1.5%), with 0.6 M KCl or 0.8 M mannitol or glucose as osmotic stabiliser at pH 6.5. Almost complete protoplast formation was attained within 2 h. After enzyme removal, protoplasts (10^8 ml^{-1}) from the two mutants were mixed in a ratio of 1:1 and 10 ml portions in the osmotic stabilisers at pH 5.5 and 7.0 were sedimented in 1 cm diameter centrifuge tubes at 2000g. The sedimented protoplasts were kept at 4 or 20° C for 12 h, resuspended, plated on osmotically stabilised minimal medium containing 2% agar and covered with a thin layer of the same agar medium by spraying. The hot agar solution (approximately 80° C) was sprayed in sterile conditions with the aid of an atomiser from the distance of about 50 cm. The temperature of the agar droplets was about 30° C at the time of reaching the surface of the inoculated plates. Slightly better results could be obtained if the surface of the plates were allowed to dry before spraying. The thickness of the covering layer was about 0.2 mm. By using the spraying method, almost 100% protoplast regeneration could be achieved with the prototrophic *Geotrichum* strain, or with auxotrophic mutants in supplemented media. Plates were incubated at 30° C and checked for nutritional complementation.

With mannitol or glucose as stabiliser, complementation occurred with low but constant frequency. At 20° C the average frequency of complementation was 1.6×10^{-6} per protoplast pair at pH 5.5, and 6×10^{-7} at pH 7. At 4° C the proportion of complemented cells was even lower. Complementation did not result with KCl as osmotic stabiliser.

No colonies appeared from separately treated protoplasts of the auxotrophic mutants; nor if arthrospores or growing hyphae of the two mutants were mixed, kept together, and plated as for protoplasts; nor if mixed but not sedimented protoplasts were plated; nor if protoplasts of one mutant were mixed with burst protoplasts from the other mutant.

Colonies derived from complemented cells differ char-

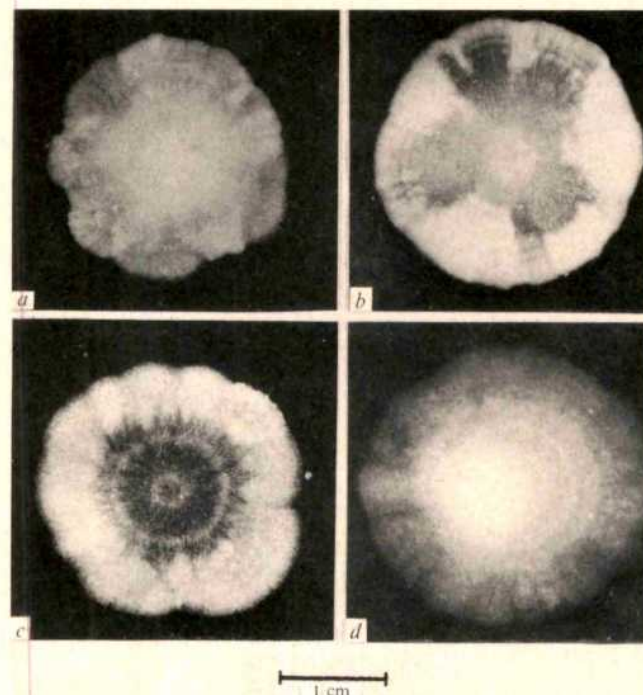


Fig. 1 Macrocolonies of *Geotrichum candidum*. a, Prototrophic strain on minimal medium; b, Heterokaryon (*ad*+*lys*) on minimal medium; c, *ad* mutant on minimal medium supplemented with $50 \mu\text{g ml}^{-1}$ adenine; d, *lys* mutant on minimal medium supplemented with $50 \mu\text{g ml}^{-1}$ lysine.

acteristically in colour and morphology from the colonies of the original prototrophic strain and those of the parent auxotrophic mutants. The prototrophic strain and the *lys* mutant are white, the *ad* mutant is red; colonies of heterokaryons are different shades of pink. The morphological differences are illustrated in Fig. 1.

Serial transfer of the complemented hyphae could be performed indefinitely using minimal medium. Complementation, however, was temporary. Complemented hyphae transplanted using media rich in adenine and lysine gave rise mainly to auxotrophic arthrospores. In agar cultures, the proportion of segregation into *ad* and *lys* mutants differed from colony to colony. Between the segregants, the *lys/ad* quotient proved always higher than 1, with the highest value of 6.5 and the lowest of 1.4. The average ratio in colonies 2 d old was 6:3:2, *lys*, *ad* and complemented arthrospores respectively. This ratio changed with time, the growing colonies bearing proportionally fewer and fewer complemented arthrospores.

Colonies developed from complemented arthrospores again bore nutritionally deficient arthrospores. Permanently complemented cells have not been observed up to the present.

From complemented hyphae grown on minimal medium, mostly complemented arthrospores were obtained, although arthrospore formation was rare, especially within the liquid. A considerable number of auxotrophic colonies, however, could be regenerated from protoplasts produced from complemented hyphae after repeated protoplast formation.

Nutritionally deficient stable mutants of *Geotrichum candidum* offer many technical advantages in studies of the conditions required for a more efficient means of fungal protoplast fusion. Further studies with *Geotrichum candidum* and other species to increase the frequency of successful fusion are in progress.

It may be mentioned that morphological alterations are not confined to *Geotrichum*; after protoplast fusion of nutritionally deficient *Aspergillus nidulans* mutants, well-defined differences can also be observed between colonies of the wild-type strain and those of the heterokaryons.

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Mechanical shocks induce phenocopies of developmental mutants

In the multicellular green alga *Ulva mutabilis* Føyn, the early division pattern and cellular differentiation can be influenced by exposing the zygotes to mechanical shocks created by a sledge-hammer. The treated plants mimic certain mutants with an abnormal division pattern and/or with less cellular differentiation than wild type. Figure 1c, and Fig. 2c, show

examples of two types of abnormal plants induced by two different strokes of the sledge-hammer, and Fig. 1a, Fig. 2a, Fig. 1b, and Fig. 2b, show corresponding stages of the wild type and two developmental mutants.

Normally the wild type starts its development by forming a filament consisting of a row of cells. When the filament is about 16 cells long, the division planes become orientated through the longitudinal axis and the germling is, essentially, transformed into a tube closed at both ends. The proximal part differentiates into a holdfast with large stem cells and elongated rhizoid cells, while the distal part flattens and expands as a sheet consisting of a double layer of polygonal cells.

A number of mutants have been isolated, which instead of the three morphologically distinct cell types of wild type contain only one or two. Some of these mutants pass a filamentous stage¹ but others do not and develop as hollow spheres² or as aggregates of cells³. At least some of these mutations seem to be in genes active during early stages of development^{4,5}.

The direction of the first division plane of wild type can be set by applying unidirectional light or an electrical field for a certain period about 24 h before the first division⁶. The division plane is then perpendicular to the vectorial stimulus and to the surface on which the cells are growing. The following planes are always parallel to the first and cannot be influenced by light or an electrical field. Filamentous growth, however, is not dependent on a vectorial stimulus of any duration, because normal development occurs when zygotes are grown in agitated suspensions. Centrifugal forces up to 280,000g or ultrasonic treatment given before the first division, do not affect development. Morphogenesis is also normal when the amount of light is reduced and growth is extremely slow.

Until now, the only treatment we have found which affects morphogenesis is exposure of the zygotes to mechanical shocks. The idea came from the work of Kamiya and Kuroda⁷, whose 'hammer experiment' succeeded in releasing *Nitella* chloroplasts from their cortical positions.

Zygotes were prepared⁸ and allowed to settle overnight on cover slides 18 × 18 mm. Thereafter, they were exposed to routine culture conditions⁴ with a light: dark cycle (0400h–1700h, light; 1700h–0400h, dark) starting about 9 a.m. The following day the glass, with hundreds or thousands of zygotes, was placed on the honed surface of a sledge-hammer (Fig. 3) and kept there by an O-ring pressed against the glass by a lid fastened with three screws. An anvil was struck with the other end of the hammer. The zygotes were then cultivated further and a clearcut effect could at the earliest be observed when the zygotes started to divide

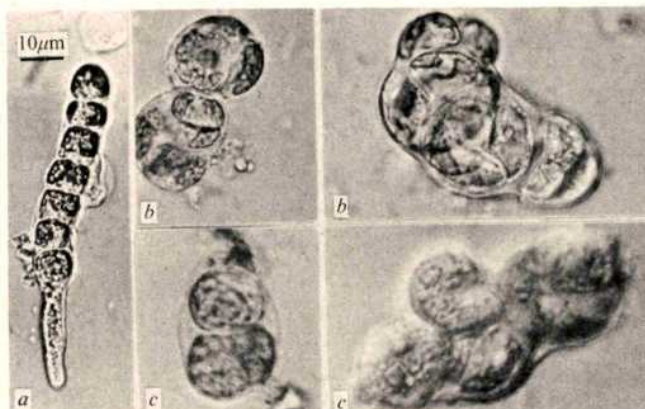


FIG. 1 a, Wild-type germling; b, two developmental stages of the mutant lumpy; c, two developmental stages of a wild-type zygote exposed to mechanical shocks at an age of 1d (2 d before the first cell division in the controls).

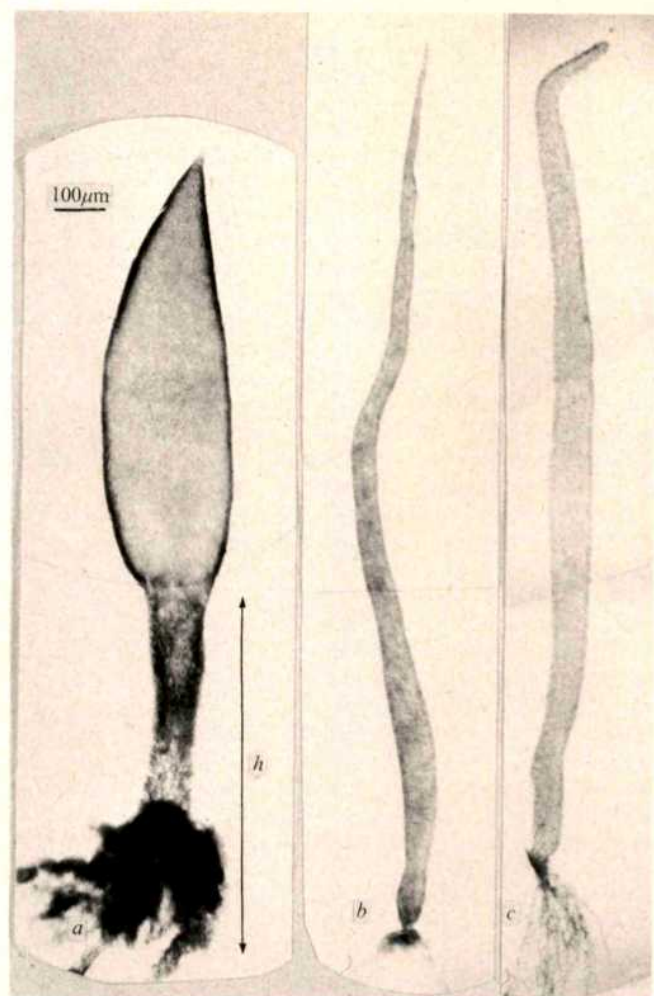


FIG. 2. *a*, Wild-type plant about 4 weeks old (*h*, holdfast); *b*, mutant 4b about 4 weeks old; *c*, wild-type plant about 4 weeks old. The plant was exposed to a mechanical shock as a 1-d-old zygote.

48–72 h later. The possibility that the effect of the stroke is created by a sudden increase in hydrostatic pressure within the O-ring is excluded, because plants outside the ring also react.

Our first experiment showed that all zygotes on a glass developed in the same way. Of 11 successive strokes, four gave normal plants, two gave plants which did not form a proper filament and showed delayed differentiation, and five gave plants which passed a filamentous stage, but did not differentiate a proper holdfast. Numerous other experiments have given similar results, and the two types of abnormal plants have been recorded repeatedly.

The first kind of abnormal development is illustrated in Fig. 1*c*. The division planes are arbitrarily arranged and the plants develop as cellular aggregates similar to those of the mutant *lumpy* (Fig. 1*b*). The walls between the cells appear curved instead of straight. Even before division, there is the impression that something is wrong with wall architecture and at high magnification the outline of the walls seems thicker and more irregular than normal. The generation time of the cells is longer than usual and differentiation is delayed for weeks if not months. The change induced by the stroke should therefore be considered heritable through many cell generations. Finally some cells regain normality and wild type plants sprout from the aggregate.

The second kind of effect is illustrated in Fig. 2*c*. The plants develop as filaments and are transformed into tubes. The proximal differentiation of the large stem cells and the flattening of the distal part does not take place. They re-

produce much earlier than wild type. The effect of the stroke can already be seen on the filaments. In wild type, the width of the cells decreases from base to apex but in the treated plants the cells are more uniform. The development of secondary rhizoids is also delayed compared to the controls, but a fair amount of rhizoid cells will develop and fasten the plant to the substratum. The abnormal plants mimic a certain type of developmental mutants (Fig. 2*b*) not previously described.

In one experiment, zygotes 1, 2, 3 and 4 d old were treated. Ten blows were struck each day, and of them five, four, three and five strokes respectively had an effect on development. There is therefore no indication of a period before the first division, when the zygotes are especially vulnerable. When the head of the sledge-hammer was dropped through an iron pipe onto the anvil from heights of 6, 12 and 18 m, three of eight, nine of eleven and four of eleven experiments gave abnormal development. The experiments were repeated with a steel cylinder, made to accommodate the zygotes in the same manner as the sledge hammer, but numerous trials from the same and even greater heights, gave no effects at all. The cylinder was a little shorter than the sledge hammer, had no hole for a shaft, but was given the same hardness as the hammer. No effects were obtained when the cylinder was fastened to a shaft by a ring-shaped holder and used as a hammer.

Our experiments show clearly that the wild-type zygotes of *Ulva mutabilis* can be induced to mimic the development of certain mutants. It is important to note that either the plants do not react at all to the treatment, or every single plant of the exposed population reacts by following a particular abnormal developmental path. It is also of significance that effects were obtained with a particular sledge hammer, and that the results could not be repeated by using one of another form. These facts raise the question if the different effects are due to different shock waves, created because one stroke is not exactly like another.

It is common opinion that the early development depends on the cytoarchitecture of the cell, especially the cortex, and it is therefore possible that the treatment distorts the cytoarchitecture of the zygotes in an irreversible manner. There is the most interesting possibility of changing the

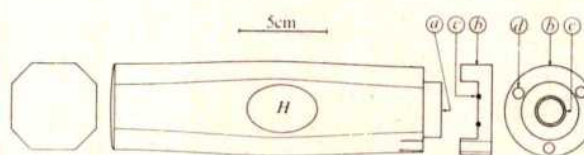


FIG. 3. The sledge-hammer with which the effects were obtained. *a*, The honed surface on which the cover-slide with the zygotes was placed; *b*, the lid with O-ring; *c*, and three screw-holes; *d*, to press the cover slide against the surface. *H*, Hole for the wooden shaft. The hardness of the steel was 68 Rockwell. The sledge hammer was made for the Municipal Tramcar Company in Oslo about 50 yr ago and specimens are no longer available.

cytoarchitecture in defined directions. Our experimental arrangements are primitive but we hope this note will bring us in contact with people who can suggest more appropriate methods, which enable measurements and calculation of the velocity, amplitude and shape of the shock wave.

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Differential survival of skin and heart allografts in radiation chimaeras provides further evidence for Sk histocompatibility antigen

CERTAIN inbred mice, lethally irradiated and inoculated with bone marrow and spleen cells from F1 hybrids, reject donor-strain skin grafts although they are permanent haemopoietic chimaeras¹⁻³. After rejection their serum is specifically cytotoxic *in vitro* for donor-strain epidermal cells but not lymphoid cells⁴, and their leukocytes are stimulated specifically in mixed cultures by donor-strain epidermal cells but not leukocytes⁵. Thus epidermal cells contain distinctive surface alloantigens (skin-specific or 'Sk' alloantigens) which have been suggested to function *in vivo* as histocompatibility antigens. If this is correct, the rejection of donor-strain skin grafts by persistent chimaeras could be explained as a loss of 'self-tolerance' to donor-strain Sk antigens by grafted haemopoietic cells as they proliferate in allogeneic recipients in the absence of these antigens^{1,2}. This interpretation is strengthened because donor-strain skin grafts are not rejected if radiation chimaeras receive donor-strain Sk antigen in the form of epidermal cell suspensions at the time of marrow restoration and thereafter⁶.

Working with inbred rat strain combinations which differ considerably in the survival of skin and organ allografts, we were attracted to the proposed Sk histocompatibility system because of its potential importance as a model of tissue-specific allograft reactions. For example, by analogy with the rejection of skin allografts in the face of persistent haemopoietic chimaerism, the rejection of skin allografts in situations where heart transplants survive indefinitely might result from alloantigens present in skin but absent from heart tissue. Alternately, the differential survival of skin and organ allografts might depend on non-specific differences in their mode of transplantation; natural vascularization and prolonged ischaemia compared with prompt surgical anastomosis, quantitative differences in graft antigenicity, different routes of sensitization and so on.

Previously we attempted to minimize non-specific differences between skin and heart transplants by grafting neonatal heart fragments subcutaneously⁷. Such allografts were rejected acutely like skin allografts, even though surgically

anastomosed heart organ allografts from the same donor strain survived indefinitely^{8,9}. Although this gave no evidence for Sk histocompatibility antigens in rats, they were not ruled out. Moreover, reports that surgically anastomosed skin allografts were rejected acutely in a rat strain combination where kidney allografts survive indefinitely^{10,11} suggested the presence of Sk antigens.

The ability (of J. S. L.) to anastomose organ transplants in mice has facilitated comparison of the survival of surgically anastomosed with naturally vascularized allografts in mouse strain combinations where Sk histocompatibility antigens are presumably operative. As in our experiments with rats, our rationale was to control the non-specific factors in grafting different tissues to assess the importance of tissue-specific factors. We now report the first results of our experiments with heart allografts in mouse radiation chimaeras that reject donor-strain skin allografts while remaining permanent haemopoietic chimaeras.

We produced Sk-reactive radiation chimaeras as described by Boyse *et al.*¹, by exposing C57Bl/6 (B6) mice to 750r whole-body X-irradiation and then inoculating them intravenously with about 50×10^6 (B6 \times A)F1 hybrid spleen cells. As controls for the effects of irradiation we inoculated other similarly irradiated B6 mice with B6 spleen cells. After 11-12 weeks we sent citrated blood samples from the presumed B6/B6 \times A chimaeras to Dr E. A. Boyse's laboratory for H-2 typing of erythrocytes¹. One to three weeks later (that is, 12-15 weeks after irradiation and cell restoration) we challenged those mice with clear evidence of B6/B6 \times A chimaerism and the B6/B6 controls with various types of strain A test grafts. All the skin grafts were transplanted orthotopically to the thorax and evaluated in terms of grossly observable epithelial survival as described by Billingham¹². Since it is necessary to use perinatal donors to obtain heart fragments that acquire assayable pulsatile activity⁷, in one series skin grafts were taken from the newborn mice that provided the heart fragments in order to control for any possible reduced immunogenicity of neonatal tissue. Moreover, 2-mm diameter "mini"-sized grafts were used with the intention of approximating the tissue antigen dosage of the heart fragments. Adult heart organs were transplanted heterotopically to the abdomen as described by Corry *et al.*¹³; newborn heart fragments were transplanted subcutaneously to the ear as described by Fulmer *et al.*⁷. Both types of heart grafts were monitored electrocardiographically as well as grossly and rejection was defined as permanent cessation of electrical activity.

Table 1 shows B6/B6 controls rejected both types of skin and heart test grafts acutely. Indeed, these survival times are similar to those for comparable test grafts in intact non-irradiated B6 recipients, indicating that there were no apparent residual effects of radiation. In contrast, there was a striking difference in the fate of the skin and heart test grafts in the chimaeras. The chronic rejection of the skin grafts verifies the observation of Boyse *et al.*^{1,2} No heart test graft

TABLE 1 Survival of skin and heart test allografts in radiation chimaeras and controls

Type of strain A test graft	Number	B6/B6 controls MST \pm s.d.*	Test graft survival (d) in:			
			Range	Number	B6/B6 \times A chimaeras MST \pm s.d.	Range
Adult donors						
skin	7	8.7 \pm 1.1	8-10	10	20.2 \pm 1.4	14-36
heart organ	8	9.0 \pm 1.4	7-12	5	(66+†, 70+†, 146†, 162†, 175†)	
Newborn (0-48 h-old) donors						
'mini' skin§	6	7.3 \pm 1.3	8-12	11	27.0 \pm 1.4	18-52
heart fragment	7	13.5 \pm 1.1	12-16	10	(All 175†)	

* Median survival time \pm s.d. as computed by Litchfield's nomograph method¹⁶.

† Died with surviving test graft.

‡ Alive with surviving test graft as of November 30, 1973.

§ 2-mm diameter as opposed to the 15-mm diameter adult skin grafts.

recipient has rejected its test graft so far, although two died with surviving grafts. Three months after skin grafting, and well after rejection of the last test graft, we again sent blood samples to Dr Boyse for tissue typing. In every case but one, chimaerism had persisted in spite of the donor-strain skin allograft rejection. Moreover, 2 months after heart grafting, all recipients, when challenged, rejected strain A skin test grafts without discernable effect on preexisting heart grafts which, as just indicated, are still surviving.

We draw two conclusions from the differential survival of skin and heart allografts in our chimaeras. First, in the strain combination tested there is no evidence that mouse heart tissue possesses distinctive histocompatibility alloantigens analogous to Sk. It would, however, be unsafe to conclude that such antigens are not present in heart cells. Even in the case of Sk antigens a serological anti-Sk response has been demonstrated in the absence of graft rejection⁴, as is also the case in the H-Y¹⁴ and Θ ¹⁵ incompatibility systems. Second, the case for Sk functioning as histocompatibility antigen is strengthened, particularly by the differential survival of the newborn skin and heart fragment allografts. Here, where a deliberate attempt was made to graft skin and heart tissue under comparable non-specific conditions (a strategy for revealing the possible contribution of tissue-specific factors), the differential survival observed with the conventional skin and heart organ allografts from the adult donors was maintained.

Our results provide further evidence the Sk antigen can function as histocompatibility antigen. However, they do not rule out an important role for non-specific factors^{8,9} in acute skin allograft rejection because the roles of tissue-specific and non-specific factors are not mutually exclusive. The only definitive way of determining the relative *in vivo* importance of anti-Sk immunity may be through the creation of a congenic resistant line of mice differing from an inbred strain only at the presumed Sk locus.

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Pharmacological evidence related to the existence of two sodium channel gating mechanisms

IN 1952, Hodgkin and Huxley¹ proposed the now classical differential equation that has become the accepted mathematical model for describing action potentials. In this equation there are three probability factors, m , n and h , which depend on transmembrane potential and time. Conceptually these probability factors are often considered to represent three field-dependent molecular gating mechanisms that mediate ionic flow through two transmembrane channels. The m and h gates mediate sodium currents and the n gate controls potassium flow. Electrical evidence has been presented for a molecular rearrangement associated with sodium activation (for example, m gate opening)². This evidence is based on the detection of transient electrical currents in the axon membrane which are hypothesised to represent rearrangement of molecular dipoles.

Pharmacological evidence for these gating mechanisms is scanty. Purified conylactis toxin³ has been reported to interfere with sodium inactivation⁴, but only in invertebrates (personal communication from B. I. Shapiro), thus restricting its usefulness and generality. Tetrodotoxin and saxitoxin are believed to bind to a common site on the sodium channel^{5,6}, but so as to decrease maximum sodium permeability, \bar{g}_{Na} , rather than affecting any of the probability factor equations. The difficulty of synthesising active derivatives of these complex alkaloids limits their usefulness as biological probes of structure-activity.

We have been investigating neurotoxins that act specifically on action potentials in vertebrates and may therefore be useful as probes of the nervous conduction mechanism in higher animals⁷. The toxins are components of the venom of the scorpion *Heterometrus gravimanus*. Two active fractions have been isolated one of which seems to delay sodium inactivation while the other exerts an effect which may be consistent with an abnormally persistent sodium activation.

Lyophilised venom of *Heterometrus gravimanus* (Sigma) was fractionated on a column of Whatman 23 CM-cellulose as shown in Fig. 1. Seven peaks led absorption above back-

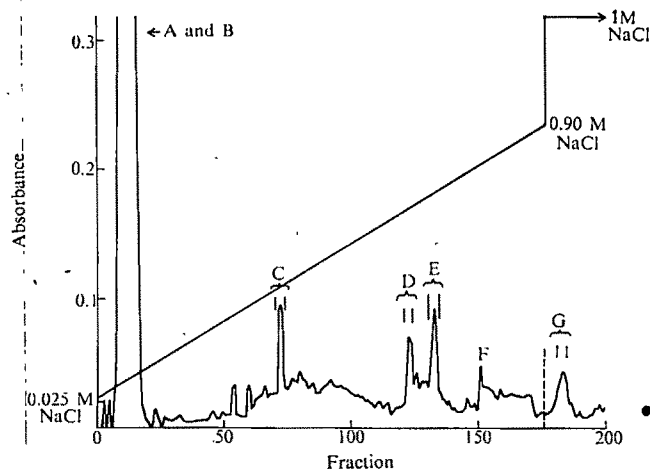


FIG. 1 Chromatogram of *Heterometrus gravimanus* venom on carboxymethyl-cellulose (Whatman 23). Absorbance was measured at 280 nm.

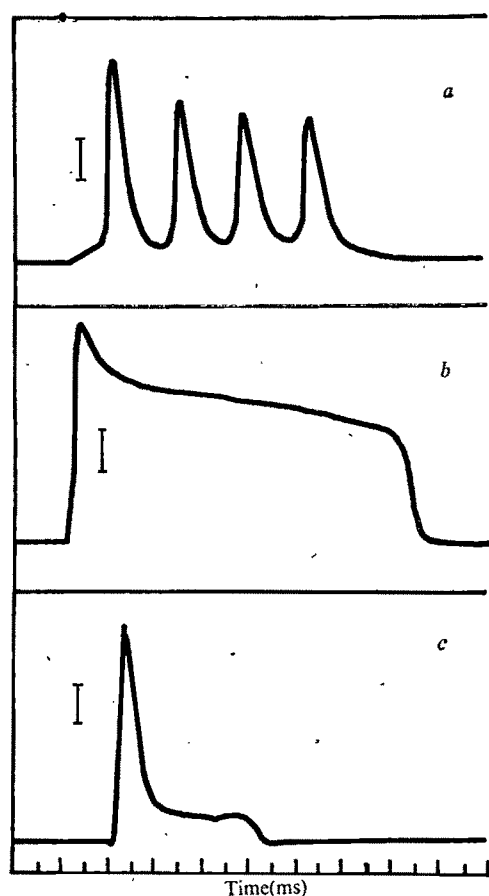


FIG. 2 Action potentials recorded at the node of Ranvier from sciatic nerve fibres of *Xenopus laevis* after application of venom or venom fractions. Procaine (1%) was used to block biphasic potentials. The vertical markers represent a scale of approximately 20 mV. *a*, Crude venom ($20 \mu\text{g ml}^{-1}$); *b*, fraction D from chromatographed venom ($1 \mu\text{g ml}^{-1}$); *c*, fraction C from chromatographed venom ($1 \mu\text{g ml}^{-1}$).

ground at 280 nm, and all peaks gave positive tests for protein by the method of Lowry *et al.*⁸ Table 1 gives this data in more detail. It is interesting that fractions C and D, the only two that affect action potentials, each represent less than 1% of the venom by weight.

Action potential responses were obtained by a technique described earlier^{9,10}, in which single medullated nerve fibres from the sciatic nerve of *Xenopus laevis* were recorded using a single air gap technique. In some experiments, 0.11 M KCl was used to block the biphasic action potential on one side of the air gap and in other experiments procaine was used. Continuous flow of fluid over active nodes in the opposite pool adjacent to the air gap was maintained relatively constant by means of gravity flow from a Mariotte flask.

When crude venom was applied to a node for several minutes in a dose of $20 \mu\text{g ml}^{-1}$, repetitive action potential spikes resulted (Fig. 2*a*). There was no long delay in the inactivation process as had been reported for the venom of *Lieurus quinquestriatus*¹¹. The effect was irreversible. However, when the venom was first dialysed against Ringer solution for 4 h and then applied to the nerve fibre, no repetitive spikes were seen, but there was a large and irreversible delay in sodium inactivation when venom was applied in a dose of $50 \mu\text{g ml}^{-1}$ (similar to Fig. 2*b*). Twenty to thirty minutes after application of venom, the delay was several hundred milliseconds. The difference between the results of experiments conducted before and after dialysis suggested that there was more than one venom component, each acting by a different mechanism. At least one of these components would be either readily dialysable or absorbed

by the membrane. Addition of 10mM tetraethylammonium chloride (TEA) to the perfusion medium failed to stop either the repetitive spikes or the sodium inactivation delay, giving further evidence that the sodium channel was the target of the venom. TEA selectively blocks potassium conductance increases¹².

Subsequent tests of fractionated venom components gave the following results. First, only fractions C and D affected the action potential. Fig. 2*b* shows an action potential from a nerve fibre poisoned by fraction D. There is an irreversible delay in sodium inactivation at concentration of $1 \mu\text{g ml}^{-1}$, similar to that observed in the crude venom of *Lieurus quinquestriatus*¹¹. With fraction C (Fig. 2*c*), however, the time course of voltage increases and decreases during the action potential was relatively unaffected until the membrane potential returned to about 20 mV above the resting level. Recovery was then characterized by a persistent plateau lasting many milliseconds. Since the initial time course of sodium inactivation seemed to be essentially unchanged, we concluded that persistent sodium activation may have been involved. This conclusion is reinforced by observations in which the crude venom of *Centroides sculpturatus* was found to prolong sodium activation¹³. Increasing the dosage or prolonging the exposure only increased the length of the trailing plateau and did not affect its potential level. TEA (10 mM) failed to abolish this plateau, although a slower spike decay was apparent, as would be expected from an action potential without the delay potassium permeability change. This effect was also irreversible.

At this stage, we have no explanation for the repetitive spikes produced by crude venom, although it may be the result of combined interactions of two or more venom components with the nerve membrane.

Positive results in the Lowry test suggested that the active components of the venom were protein. Preliminary studies on sodium dodecyl sulphate gel electrophoresis indicated molecular weights of less than 10,000, and the high NaCl molarity required to elute these fractions from the CM-cellulose indicated a positively charged protein. These properties are reminiscent of those possessed by the cholinergic neurotoxins of Elapid snake venoms. (Detailed biochemical studies will be reported later.)

TABLE 1 Protein content of each peak estimated by Lowry's method

Fraction	Absorbance (750 nm)	Est. conc. ($\mu\text{g/ml}^{-1}$)	% of Venom (weight)
A	>0.500	>140	>7.0
B	0.329	90	3.6
C	0.115	33	0.66
D	0.170	48	0.96
E	0.152	43	1.29
F	0.150	43	0.43
G	0.249	71	2.85

The instrument for measuring absorbance was calibrated with bovine serum albumen as standard.

Our results may be significant for two reasons. First, they offer possible pharmacological evidence, for two molecular gating mechanisms that may be a biochemical correlate of the *m* and *h* probability factors originally derived by Hodgkin and Huxley¹. Second, and perhaps more important, these neurotoxins have potential application as previously unavailable biochemical probes of the sodium channel. A parallel situation existed with the cholinergic neurotoxins of the Elapid snake venoms in that they allowed the first widely acceptable isolations and partial biochemical characterisations of the acetylcholine receptor.

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Absorbance changes induced by blue light in *Phycomyces blakesleeanus* and *Dictyostelium discoideum*

BLUE and near ultraviolet light controls several metabolic and physiological processes in organisms ranging through bacteria¹, fungi² and algae^{3,4} to vascular plants⁵ and animals^{6,7}. The similarity of the action spectra of these various light-induced responses (all have action maxima at about 470 nm) suggests a common photoreceptor pigment system which controls these different cellular processes. We have looked for light-induced absorbance changes that might be related to such a pigment system.

Our initial work involved *Phycomyces blakesleeanus* because extensive investigations had been made of the blue-photoreceptor pigment in this organism². (This pigment resides both in the sporangiophores where it controls the phototropic response² and in the mycelium where it controls sporangiophore initiation⁸.) Mycelial mats were used rather than sporangiophores because of the greater ease of collection and preparation, and because the large vacuoles in the sporangiophores made them less promising material for spectrophotometry. We also used cell suspensions of *Dictyostelium discoideum*.

Light-minus-dark difference spectra were measured with a single-beam spectrophotometer on line with a PDP8/I computer. The absorption spectrum of the sample was measured (20 s per scan) just before and 30 s after a 30 s irradiation with actinic light obtained with a xenon lamp in conjunction with interference filters (12 nm half-bandwidth). The same timing sequence was used between irradiation and measurement in all experiments. The kinetics of decay of the light-induced absorbance changes after irradiation were measured with an Aminco-Chance dual-wavelength spectrophotometer.

The light-minus-dark difference spectrum (that is, the difference between the absorption spectrum measured after irradiation and that measured before irradiation) of a fresh

sample of *P. blakesleeanus* due to irradiation with 470 nm actinic light shows bleaching at 445 nm and an increase in absorbance at about 430 nm (Fig. 1a). If the sample was allowed to become anaerobic in the cuvette before irradiation, there was no bleaching at 445 nm but the absorbance increase at 430 nm remained (Fig. 1b). Both these absorbance changes decayed in darkness with a half-time of about 70 s.

Blue light at about 470 nm contains the most effective wavelengths for eliciting these absorbance changes. These wavelengths are also the most effective for the physiological blue light responses of *P. blakesleeanus*². Dense mycelial mats, however, are not suitable for measurement of an action spectrum of the photosensitive absorbance change; the response is not sufficiently stable for repetitive excitation of a given sample with different wavelengths and the preparation techniques are not sufficiently reproducible to permit fresh samples for each actinic wavelength.

Berns and Vaughn⁹ reported different light-induced absorbance changes with mycelial mats of *P. blakesleeanus*: irradiation with 345 nm actinic light caused an absorbance change between 460 and 345 nm (which could have been due to bleaching at 345 nm or to an increase in absorbance at 460 nm) of about 0.04 which persisted for several minutes. We tried to reproduce these measurements using a filter double-beam spectrophotometer comparable with that used by Berns and Vaughn, but found no light-induced absorbance differences between 460 and 345 nm greater than the noise level of the measurement (0.002).

Cell suspensions of *D. discoideum* were also examined for light-induced absorbance changes. The light-minus-dark difference spectrum of a fresh sample of cells taken near the end

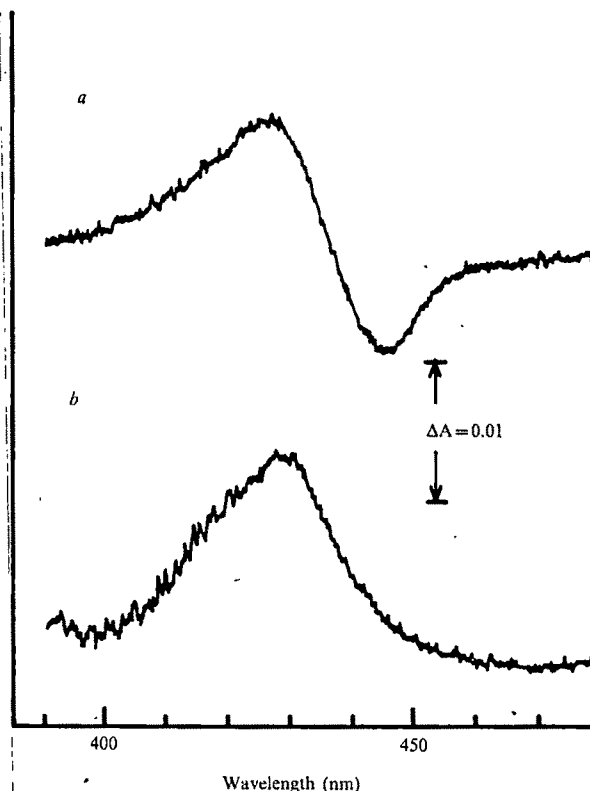


FIG. 1 Light-induced absorbance changes in *P. blakesleeanus*. A carotenoid deficient strain of *P. blakesleeanus* (C-2) was grown for 5 d in stationary culture after inoculation with heat-shocked spores of the medium described by Carlile¹³. The mat of mycelium was lifted from the culture flask and plugs were cut with a cork borer to fit the bottom of a cylindrical vertical cuvette. The sample was about 3 mm thick and represented an absorbance of about 3 at 425 nm. Actinic light was 3 mW cm⁻² at 470 nm. *a*, Light-minus-dark difference spectrum of a freshly prepared sample. *b*, Light-minus-dark difference spectrum of a sample permitted to go anaerobic in the cuvette before the measurements.

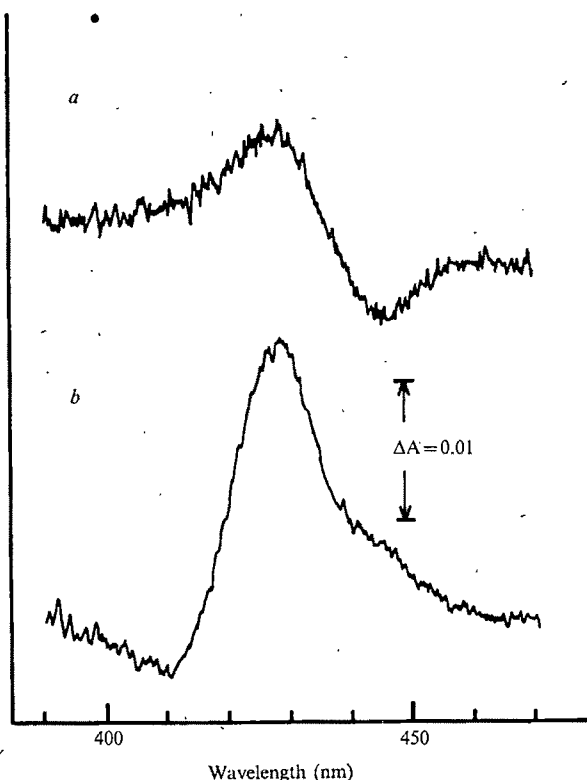


Fig. 2 Light-induced absorbance changes in *D. discoideum*. A strain A3 of *D. discoideum* able to grow in axenic culture was grown in liquid medium and collected as previously described¹⁰ while in late exponential growth phase. The sample consisted of a cell suspension (4×10^8 cells ml⁻¹) approximately 3 mm thick which represented an absorbance of about 3 at 425 nm. *a*, Light-minus-dark difference spectrum of a freshly prepared sample. *b*, Light-minus-dark difference spectrum of a sample permitted to age for several hours before the measurements.

of log phase growth (Fig. 2*a*) shows bleaching at 445 nm and an increase in absorbance at about 430 nm. If the cells aged for several hours before the experiment, there was no bleaching at 445 nm and the increase in absorbance at 430 nm was generally somewhat greater than without ageing (Fig. 2*b*). In the latter case, slight bleaching was also observed near 410 nm. The light-induced absorbance changes in cells of *D. discoideum* (Fig. 2) were essentially the same as those in the mycelium of *P. blakesleeana* (Fig. 1).

Two different light-induced absorbance changes can be measured in cells of *D. discoideum*: one is a small rapidly decaying absorbance increase at 410 nm which can be induced by wavelengths as long as 600 nm¹⁰; the other is a larger, more slowly decaying absorbance increase at 430 nm (Fig. 2) which is induced only by wavelengths shorter than 520 nm. The small absorbance increase (about 0.001) at 410 nm, induced when cells of *D. discoideum* are irradiated with blue as well as yellow light, did not contribute to the difference spectra shown in Fig. 2. The small absorbance change decays in the dark with a half-time of 7 s and thus decayed to a negligible level during the 30 s between the irradiation and the start of the spectral scan. The light-induced absorbance changes shown in Fig. 2 for *D. discoideum* cells decay in the dark with a half-time of about 70 s, as did the similar changes in *P. blakesleeana*, and thus persist long enough to be measured by the spectral scan after irradiation. The pigment which responds to the longer wavelengths of light has been extracted from *D. discoideum* cells and purified as a high molecular weight haem protein¹¹. This pigment appears to be the photoreceptor pigment for the photoactive response of *D. discoideum*. The pigment which responds only to the shorter wavelengths of light ($\lambda < 520$ nm) has been observed

both in cells of *D. discoideum* (Fig. 2) and in the mycelium of *P. blakesleeana*. Its physiological function in *D. discoideum* is unknown (there may be photoresponses controlled by the blue-photoreceptor pigment which we are not aware of in *D. discoideum*) but it is a promising candidate for the blue-photoreceptor pigment which is manifest in so many different organisms.

An approximate action spectrum for the light-induced absorbance change in aged cells of *D. discoideum* (Fig. 2*b*) was determined by measuring the absorbance change induced by equal intensities (3 mW cm⁻²) of monochromatic actinic light. A plot of the magnitude of the light-induced absorbance change as a function of the wavelength of actinic light (Fig. 3) shows that the action is maximal at about 465 nm and absent beyond 520 nm. This action spectrum for the light-induced absorbance increase at 428 nm is consistent with those for the various physiological responses which are attributed to the blue-photoreceptor pigment.

If cells of *D. discoideum* are broken gently in a Dounce homogenizer, the photoresponsive pigment sediments with the mitochondrial fraction collected by differential centrifugation between 6,000 and 10,000*g*. If the cells are broken more thoroughly by passage through a Ribi press, the photoresponsive pigment is found in the soluble supernatant from a 1 h centrifugation at 144,000*g*. The absorption spectrum of the soluble extract after cell breakage in the Ribi press (Fig. 4, curve D) shows primarily reduced cytochrome *c*. The spectrum measured after irradiation (curve L) shows small light-induced changes which are seen best at higher sensitivity in the L-D difference spectrum. The light-induced absorbance changes in the soluble extract include an absorbance increase in the 560 nm region and a larger, sharper increase at 425 nm, both indicative of a photoreduction of a *b*-type cytochrome.

The photoactive pigment system apparently consists of a photoreceptor pigment, possibly a flavin, which absorbs maximally at about 465 nm, and a photoresponsive pigment, a *b*-type cytochrome, which increases in absorbance maximally at 428 nm *in vivo* and at 425 nm *in vitro*. In the fresh tissue the photoreceptor pigment may also induce bleaching at 445 nm which could be due to the oxidation of an *a*-type cytochrome. The proposed flavin-mediated photoreduction of a *b*-type cytochrome in soluble extracts suggests a cyto-

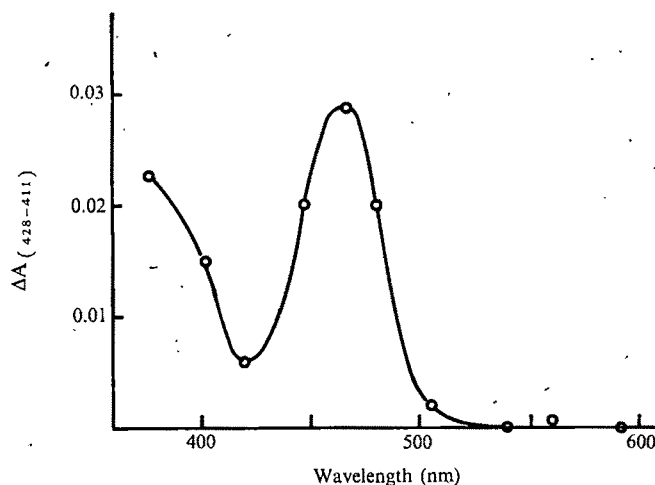


Fig. 3 Action spectrum for the light-induced absorbance change in *D. discoideum*. Aged samples were prepared as described in Fig. 2. The cells were irradiated with equal energies (3 mW cm⁻²) of monochromatic actinic light and the light-minus-dark difference spectra were recorded for the different wavelengths of actinic light. A fresh sample of cells was used for each actinic wavelength since the response tends to decay with repeated excitation. The magnitude of the light-induced absorbance change measured between the peak at 428 nm and the minimum at 411 nm is plotted as a function of the wavelength of actinic light.

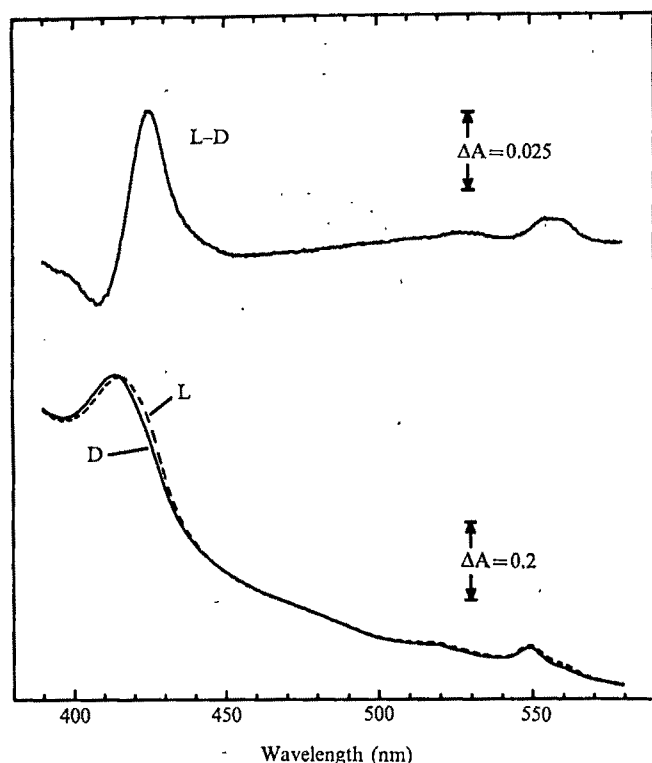


Fig. 4 Light-induced absorbance change in a cell-free extract of *D. discoideum*. Cells were grown as described in Fig. 2 to maximum stationary phase of growth at 1.2 to 1.6×10^7 cells per ml, washed, and concentrated to 10^8 cells per ml by centrifugation. The cells in this suspension were broken in a Ribi press at 12,000 pounds inch $^{-2}$ and the subcellular particles eliminated by centrifugations at 1,000g for 5 min and 12,000g for 30 min. The soluble supernatant was then collected from a centrifugation at 144,000g for 60 min. Spectra of this soluble supernatant were measured at 22° C in darkness (D) and immediately after exposure to 3 mW cm $^{-2}$ actinic light at 470 nm (L). The light-minus-dark difference spectrum (L-D) is also presented at an eight-fold increase in sensitivity (plotted directly by the computer).

chrome b_2 -like pigment with the flavin and haem prosthetic groups on the same protein molecule 12 . These speculations remain to be confirmed but the experimental system is available.

We have not obtained a cell-free system from *P. blakesleeana* which shows the photoinducible absorbance changes but these experiments are continuing. We believe, however, that a common blue-photoreceptor pigment is present in widely diverse types of cells and that cells of *D. discoideum* may be a fortuitous source of the pigment.

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Actin in the alga, *Chara corallina*

ACTIN and myosin from muscle combine to form filaments with a distinctive arrowhead appearance 1 . Filaments from several lower organisms $^{2-5}$ and from cells other than muscle in higher animals $^{6-9}$ also react with muscle myosin or its subfragments to produce arrowhead filaments. This ability to bind muscle myosin has become accepted as a test for the identification of the filaments as actin. Its validity is supported by the chemical similarities between those actins which have been further characterised $^{6,8-13}$, and by the need for the myosin binding sites to be quite specifically positioned and oriented for the arrowhead effect to arise 14 . A filament reacting with heavy meromyosin subfragment one (S_1) has now been detected in cells of the green alga, *Chara corallina*.

Internodal cells about 70 mm in length were centrifuged in a bench centrifuge at 600g for 5 min. Such treatment

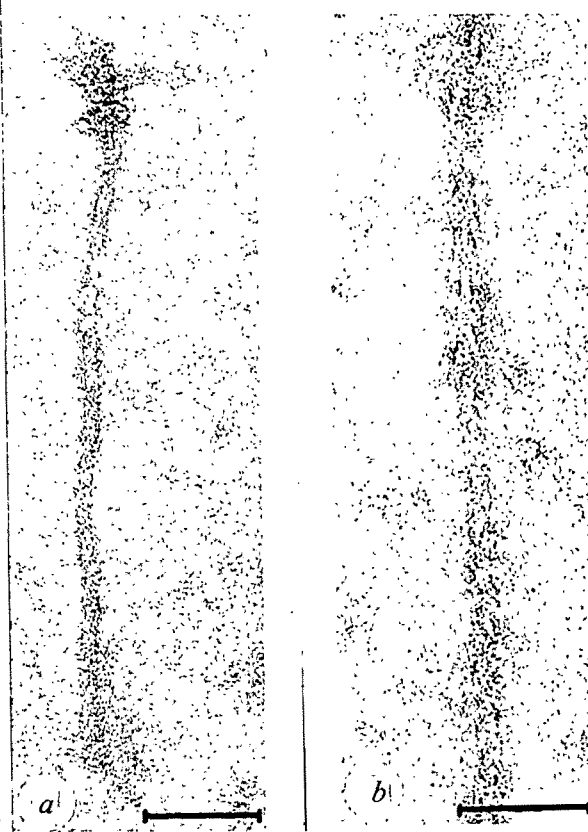


Fig. 1 a, A simple filament without S_1 treatment or, as in this case, after treatment with S_1 and ATP. b, Filament after treatment with S_1 . In places an arrowhead effect is seen; elsewhere the surface of the filament is irregular. Bar = 100 nm.

displaces the flowing endoplasm to the centrifugal end of the cell, which can then be cut off and discarded. The remainder of the cell (that part containing stationary cortical cytoplasm only) was squeezed to expel its contents. The contents of 12 such centrifuged cells were pooled and spun at 200g for 2 min. The pellet was resuspended in 50 μ l of 150 mM KCl, 10 mM trisodium EDTA, buffered at pH 7.0 with 10 mM morpholinopropane sulphonic acid. After 10 min at room temperature, the preparation was again spun at 200g for 2 min and the pellet discarded. In some experiments, an S_1 preparation from rabbit skeletal muscle was added either at the resuspension stage or to the final supernatant. The mixture was then kept at room temperature for between 10 and 60 min. All preparations were negatively stained with 1% uranyl acetate on carbon-coated specimen grids.

In the absence of the S_1 preparation, the supernatant contained simple filaments of the type shown in Fig. 1a. After S_1 treatment, filaments were found which in places had the distinctive arrowhead appearance of the actomyosin complex (Fig. 1b). If 1 min before transfer to the grid, neutralised ATP was added to a final concentration of 1 mM, the arrowhead effect disappeared (Fig. 1a). No arrowhead filaments could be found when the S_1 preparation was examined alone.

Compared with the results obtained in parallel experiments with rabbit actin, the algal filaments showed, in so far as it could be judged, less regular arrowheads. Lengths of filament without arrowheads showed an irregular, roughened surface (Fig. 1b), again quite distinct from their appearance without S_1 or after ATP treatment. Moreover, whereas stored S_1 retained its ability to decorate rabbit actin, fresh S_1 was needed to give convincing decoration of the algal filaments. These two observations may be a reflection of differences in S_1 -binding ability between the algal and rabbit filaments, although further studies will be needed to establish this.

For the reasons summarised above, the formation of such arrowhead complexes is a preliminary, but probably reliable indicator of the presence of actin. The present experiment does not settle its position or function in these cells. In this connection however, microfilament bundles are known in closely related algae¹⁵⁻¹⁷, as well as in the cells of higher plants¹⁸. Unpublished light and electron microscope observations of cells of *C. corallina* have shown that the microfilament bundles are retained with the cortical cytoplasm on centrifugation. These microfilament bundles are believed to be involved in the mechanism of cytoplasmic streaming, and could well be the location of actin within these cells.

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Photoeffects in a flavin-containing lipid bilayer membrane and implications for algal phototaxis

FLAVINS have been implicated as receptor pigments in various plant photoresponses, such as phototropism¹, phototaxis² and chloroplast movement³. It has not yet been possible to prove conclusively the flavin photoreceptor hypothesis in any of these cases, nor has a mechanism been demonstrated by which a flavin could convert a light stimulus into a physiological signal. For the light-induced motor response involved in phototaxis of *Euglena*, we have suggested an electrical type of stimulus transduction by a flavin receptor pigment embedded in a lipid matrix⁴.

We report here light-induced electrical effects in an artificial bilayer membrane of the Mueller-Rudin type⁵ that contains an amphipathic riboflavin derivative. Riboflavin tetrapalmitate was prepared according to the method of Yagi⁶. It was incorporated into an oxidised cholesterol bilayer⁷ by adding it to the membrane-forming solution at a ratio, in the bulk phase, of 1 flavin molecule to 10 cholesterol molecules. The solutions of the aqueous phases (phosphate and citrate buffers or KCl, all 0.1 M) were made up from triply distilled water and ACS grade chemicals. Illumination was with white light from a 500 W tungsten projector lamp and was passed through glass heat filters, at a maximum intensity of 4 kW

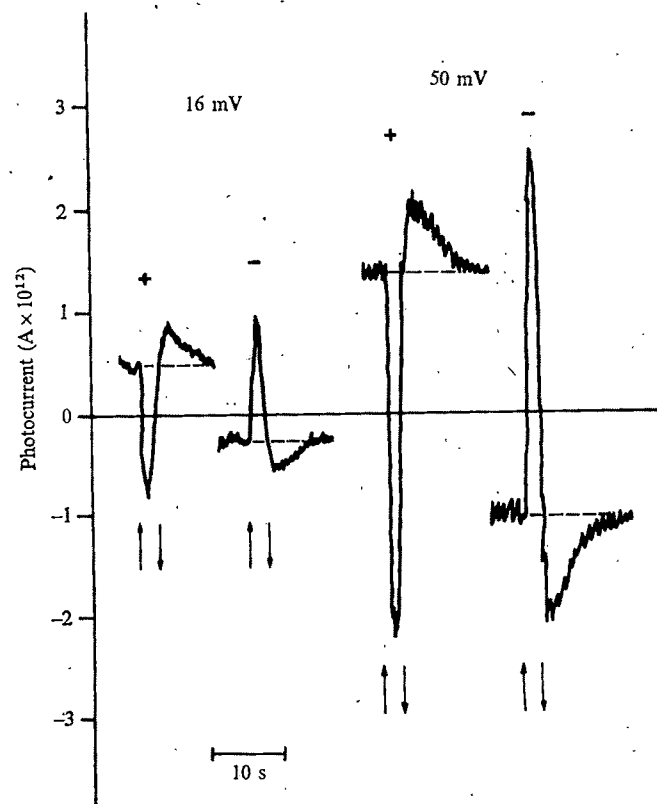


Fig. 1 Photocurrent observed as a function of applied potential on illumination of a bilayer lipid membrane containing riboflavin tetrapalmitate with white light (intensity 4 kW m⁻²).

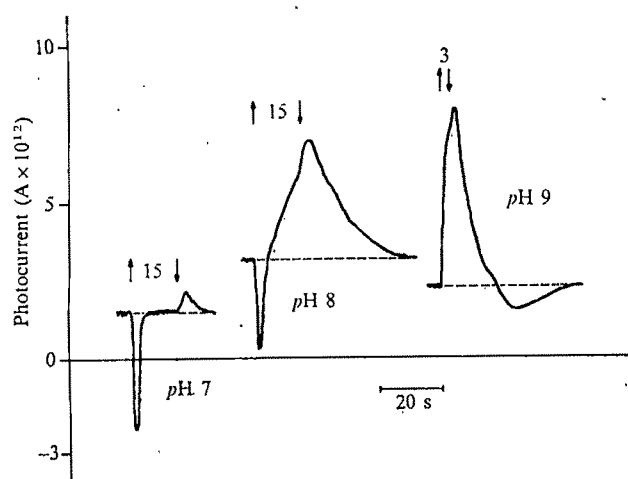


Fig. 2 Photoeffects in the flavin-bilayer membrane as a function of the pH of the aqueous phase.

m^{-2} ($4 \times 10^6 \text{ erg cm}^{-2} \text{ s}$). The photocurrent was recorded with a Honeywell Elektronik 16 strip chart recorder connected to a Keithley 610C electrometer.

Incorporation of the riboflavin tetrapalmitate did not affect the membrane resistance at neutral and slightly acidic pH, and decreased it by a factor of two to three at basic pH values. When normalised to the same peak height, photoresponses observed under the same conditions were very similar, though the magnitude of individual responses fluctuated greatly. We have not yet been able to pinpoint the reason for these variations.

As in all other systems studied so far, no photoeffects occur under completely symmetrical conditions. When an external potential was applied, at pH 6 and 7 we observed the effects shown in Fig. 1. The photoresponse consisted of a transient current decrease on illumination, followed by a return to the baseline and, when the light was turned off, a transient current pulse in the positive direction. This cannot be explained simply as an effect on membrane resistance, since the 'on'-current crosses the zero line and inverts direction transiently. The effect was the same in buffered and in unbuffered solution. From 16 to 70 mV, the peak heights of the response increased linearly with the applied potential.

As Fig. 2 shows, the nature of the photoeffect changed with increasing pH. At pH 8, the transient pulses when the light was turned on and off were still visible, but superimposed on this effect was an increase of the current lasting as long as the illumination, and slowly decreasing to the dark value after the 'off'-pulse had decayed. At pH 9 or higher, the negative 'on'-pulse was no longer discernible. In its place we observed a very rapid increase of current. The 'off'-pulse was still visible and followed by a transient slow decrease of the current below the dark value. At this pH, illumination for more than 5 s irreversibly decreased the membrane resistance.

Because of the above observation, and since the addition of flavins at pH values above 7 increases the current across the membrane even before illumination, we consider it

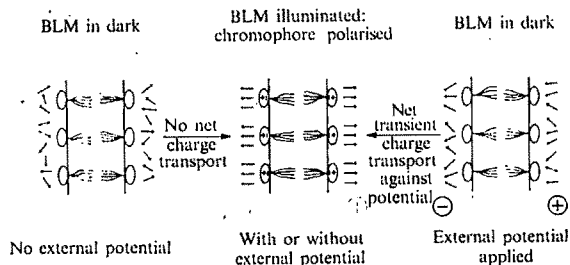


Fig. 3 Model proposed for the generation of the transient photocurrents at pH 7. BLM, bilayer membrane.

possible that the photoeffects appearing at basic pH values were, at least partly, due to effects on the membrane resistance. We have not yet attempted to substantiate this assumption.

We offer the following preliminary hypothesis to account for the characteristics of the photoeffects at neutral and slightly acidic pH. Illumination polarises the flavin moiety of the chromophore, perhaps through an electron acceptor/donor reaction of an electronically excited state of the flavin with another molecule in the lipid or aqueous phase. The polarised chromophore now interacts electrostatically with water dipoles at the membrane surface that had been pre-oriented by the applied potential. This reorientation of the dipoles corresponds to a transient transport of charges against the applied potential (Fig. 3).

This model accounts for the following observations. (1) The current pulses were transient, and a pulse in the direction of the applied potential appeared when the illumination was removed, that is when the reorienting force was released. (2) No photoeffect was observed without an externally applied potential, and reversal of the polarity of the applied potential changed only the sign of the photoresponse. (3) Increased applied potential increased the magnitude of the photoresponse, since more complete preorientation of the dipoles increased the number of charges which were transported on reorientation. At 70 mV, which was the largest potential we used because of membrane instability at higher

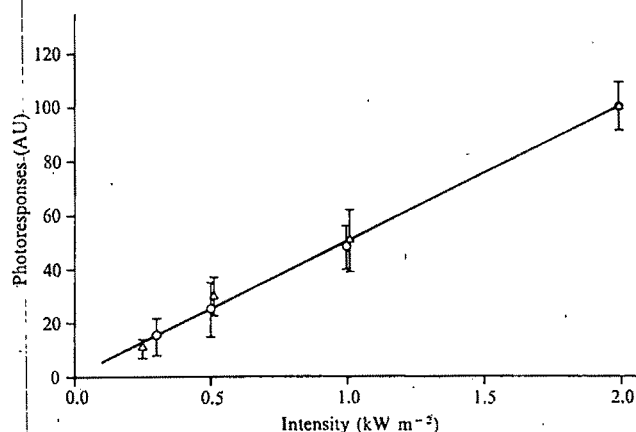


Fig. 4 Light intensity dependence of the flavin-bilayer membrane photoeffects at pH 7 (Δ) and the direct photophobic response of *Euglena* (\circ). The magnitude of both responses was arbitrarily set equal to 100 at the maximum intensity of 2 kW m^{-2} . AU, arbitrary units.

potentials, we had apparently not yet approached saturation effects caused by complete preorientation.

Calculations indeed predict that the triplet excited state of riboflavin tetrabutylate should exhibit a dipole moment which is larger than that of the ground state by a factor of 1.47 (ref. 8).

Our model is similar to that proposed by Ullrich and Kuhn for cyanine dyes adsorbed to one side of a bilayer membrane⁹. It is probably incomplete in that it cannot account for the fact that the 'off'-pulse never reaches the expected same magnitude as the 'on'-pulse. One must assume that an irreversible reaction, probably a photodecomposition of the flavin, occurs in parallel with the dipole orientation process. In agreement with this reasoning, we have observed that under all experimental conditions the photoeffects become smaller when illumination is repeated.

The interpretation of our findings with respect to the mechanism of stimulus transduction in *Euglena* must be speculative, since we have as yet no *in vivo* electrophysiological data. We have suggested previously that in *Euglena*, a physiological signal is generated by means of photoconduction

across a poised oxidation/reduction system. We observe in our bilayer membrane that photoeffects occur with an unbuffered ascorbate gradient across the membrane, but these effects disappear upon buffering. Apparently, the effects are in this case dependent upon a pH gradient rather than a redox gradient. In this context one might note that the acidities of the excited states of flavin have been calculated to considerably exceed that of the ground state¹⁰. Even though this prediction has not yet been tested experimentally, light-induced release of protons might thus provide an alternative mechanism for generation of the photocurrents observed by us.

The light intensity dependence of the bilayer membrane photoresponse at pH 7, and that of the direct photophobic response which *Euglena* exhibits when illuminated¹¹ are identical up to an intensity of 2 kW m⁻² (Fig. 4). This agreement may be fortuitous. At 4 kW m⁻² (a light intensity that we have not reached in measurements of *in vivo* responses of *Euglena*), the magnitude of the bilayer membrane photoeffect falls below an extension of the straight line in Fig. 4.

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Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid

L-GLUTAMIC acid is probably an excitatory transmitter of major significance in the mammalian central nervous system¹. The L-glutamic acid molecule is relatively flexible, and in an attempt to gain some insight into its possible shape(s) during activation of receptors associated with excitation of central neurones, a study was made of four conformationally restricted analogues: (±)-*cis*-1-aminocyclohexane-1,3-dicarboxylic acid ('cyclo-glutamic' acid), ibotenic acid, kainic acid and its dihydro derivative.

Ibotenic acid, an isoxazole isolated from the mushroom *Amanita muscaria*², and kainic acid, a pyrrolidine derivative isolated from the seaweed *Digenea simplex*^{3,4}, have previously been shown to excite central neurones^{5,6}. 'Cyclo-glutamic' acid is an inhibitor of glutamine synthetase⁷. In each of these cyclic compounds the ring restricts rotation about bonds equivalent to the C_α-C_β and/or C_β-C_γ bonds in L-glutamic acid.

The experiments were carried out on spinal interneurones and Renshaw cells of eight cats anaesthetised with pentobarbitone sodium (35 mg kg⁻¹ given intraperitoneally), administering the amino acids electrophoretically from seven barrel micropipettes⁸. Kainic acid, DL-homocysteic acid and L-glutamic acid were purchased from Calbiochem (Los

Angeles). Dihydrokainic acid was prepared by catalytic hydrogenation of kainic acid³ and N-methyl-D-aspartic acid was prepared by methylation of D-aspartic acid⁹. The micropipettes contained the following solutions: 'cyclo-glutamic' acid (1 M, adjusted to pH 7.3 with NaOH), dihydrokainic acid (0.2 M, pH 7), L-glutamic acid (0.5 M, pH 8), DL-

TABLE 1 Approximate potency ranges of excitant amino acids relative to L-glutamic acid, based on the ejecting currents required to achieve equal increases in firing frequency of spinal neurones

Amino acid	Potency range relative to that of L-glutamic acid
Kainic acid	8-80(12)*
N-Methyl-D-aspartic acid	7-18(3)
DL-Homocysteic acid	3-6(6)
Ibotenic acid	2-7(6)
L-Glutamic acid	1(25)
'Cyclo-glutamic' acid	0.7-0.8(3)
Dihydrokainic acid	0.06-0.6(5)

* Number of neurones tested.

homocysteic acid (0.2 M, pH 7.5), ibotenic acid (0.2 M, pH 7), kainic acid (0.1 M, pH 8) and N-methyl-D-aspartic acid (0.1 M, pH 8).

The approximate relative potencies (Table 1) of the amino acids as excitants of neuronal firing were assessed on the basis of results obtained with 17 interneurones and ten Renshaw cells. Kainic acid was more potent than N-methyl-D-aspartic acid, previously the most potent amino acid excitant reported¹⁰. On the other hand, dihydrokainic acid was the weakest excitant of the series. Figure 1 illustrates the effect of kainic acid on the firing rate of an interneurone in comparison with that of L-glutamic, DL-homocysteic and N-methyl-D-aspartic acids. The actions of kainic and N-methyl-D-aspartic acids were relatively slow in both onset and offset, compared to that of L-glutamic acid; this was also apparent with 'cyclo-glutamic', dihydrokainic and ibotenic acids.

An analysis of molecular models indicates that there is a unique juxtaposition of the equivalent ionisable groups in 'cyclo-glutamic', L-glutamic, ibotenic and kainic acids in the

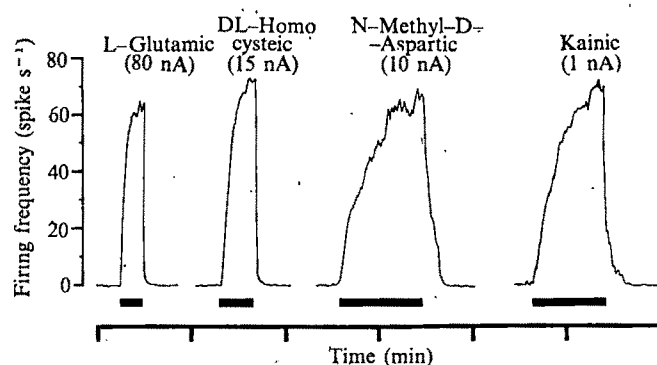


Fig. 1 Comparisons of the excitatory action of L-glutamic acid, DL-homocysteic, N-methyl-D-aspartic and kainic acids on a spinal interneurone. The black horizontal bars indicate times of electrophoretic ejection using the currents indicated (nA).

conformations illustrated in Fig. 2. It therefore seems possible that these amino acids interact with the same receptors. L-Glutamic acid is shown in a partially extended conformation with the C_α-C_β substituents eclipsed and the C_β-C_γ substituents staggered; this and the conformations shown for ibotenic and kainic acid would be expected to be relatively stable forms. The boat form of 'cyclo-glutamic' acid would be, however, less stable than a chair form. The

double bond in kainic acid must play a key role as saturation of this bond yields dihydrokainic acid which is much less potent than the parent compound as an excitant. This double bond, the conformation of which is arbitrarily illustrated in Fig. 2, might bind to a specific site on the receptor or might influence the preferred conformation and/or electronic properties of the neighbouring carboxyl group.

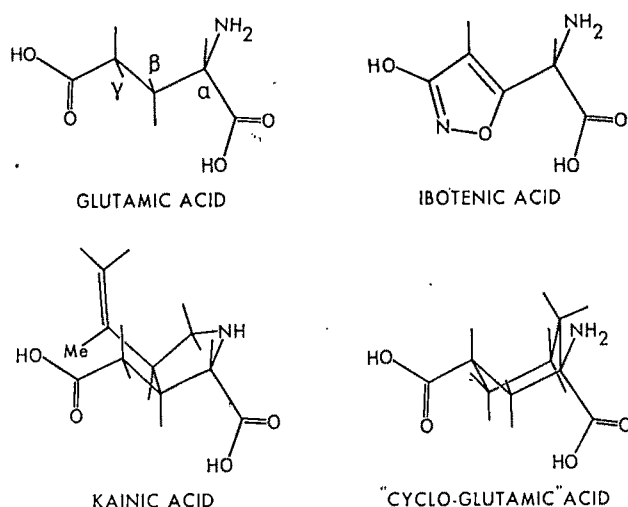


Fig. 2 Conformations of glutamic, ibotenic, kainic and 'cyclo-glutamic' acids in which the ionisable groups occupy the same absolute positions.

The ionisable groups of L-aspartic acid, also probably an important excitatory transmitter in the spinal cord¹, and of N-methyl-D-aspartic acid cannot match those illustrated in Fig. 2 for L-glutamic acid and its analogues. It is thus proposed that excitation by L-glutamic acid and L-aspartic acids results from preferential interaction by these two amino acids with different receptors. L-Glutamic acid might interact with 'glutamic acid preferring' receptors in partially extended conformations (approximating that shown in Fig. 2), and perhaps also with 'aspartic acid preferring' receptors in partially folded conformations. On the other hand, L-aspartic acid may interact only poorly, or not at all, with 'glutamic acid preferring' receptors because the carbon chain of L-aspartic acid is one atom shorter than that of L-glutamic acid.

The proposal provides one explanation of the differences in the relative potencies of L-glutamic and L-aspartic acids as excitants of Renshaw cells and dorsal horn interneurons^{2,3}. These neurones may have different populations of receptors for excitant amino acids, as a result of innervation of the interneurons, but not the Renshaw cells, by primary afferent fibres likely to release L-glutamic acid as a transmitter⁴.

Further studies with conformationally restricted analogues of L-glutamic acid, which may have different affinities for 'aspartic acid preferring' receptors compared to L-glutamic acid, may aid in assessing whether there is overlap between 'glutamic acid preferring' and 'aspartic acid preferring' receptors, though the discovery of a selective antagonist for either receptor would be of more value.

Ibotenic acid and 'cyclo-glutamic' acids were gifts from Dr C. H. Eugster (Zürich) and Dr A. Meister (New York) respectively.

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Atmospheric pressure during incubation and post-hatch behaviour in chicks

DOMESTIC chicks are widely used as convenient experimental animals, but their use has a major drawback; one batch is frequently quite unlike another. Chicks hatching one week may, for example, be behaviourally sluggish whereas chicks hatching another week may be hyperactive. Striking differences have been found in a wide variety of behavioural and biochemical measures¹⁻³.

Some batch variation may be attributable to genetic differences in the parents but marked variability is still obtained when eggs are taken from the same flock. The quality of the eggs may change as the hens grow older but this factor could hardly explain the great fluctuations in responsiveness from one week to the next. Differences might be due to variation in the time between laying and incubation of the eggs. Batch differences, however, persist when this time is kept constant and all the eggs are kept at the same temperature before incubation.

The weather is, however, one factor which does change markedly and uncontrollably from week to week. While temperature and relative humidity are unlikely to be responsible for inter-batch variation, since they are kept within narrow limits during incubation, atmospheric pressure is not usually controlled and might be an important variable. I have therefore examined the association between atmospheric pressure and a variety of measures obtained in a long experiment on imprinting in which, for many weeks domestic chicks were incubated, reared and tested under the same conditions. The results of the analysis suggest that the chick embryo is, indeed, sensitive to atmospheric pressure at a certain stage in its development.

Ten batches each consisting of 90 fertile eggs from a broiler strain (Ross Chunkies) were collected at intervals from Ross poultry Products at Fornham All Saints, Suffolk. The eggs were incubated in a Western Turkeyette incubator kept at 37.5-37.7° C. The temperature was controlled by a sealed mercury contact thermometer. The eggs were transferred 18-19 d after the onset of incubation to a still-air Curfew incubator where they hatched. Throughout incubation eggs were kept in the dark and were in contact with other eggs^{4,5}. The chicks also hatched in the dark and were kept in a dark incubator until the time of the experiment. The mean post-hatch ages at the time of the experiments and the numbers of chicks used in each experiment are shown in Table 1.

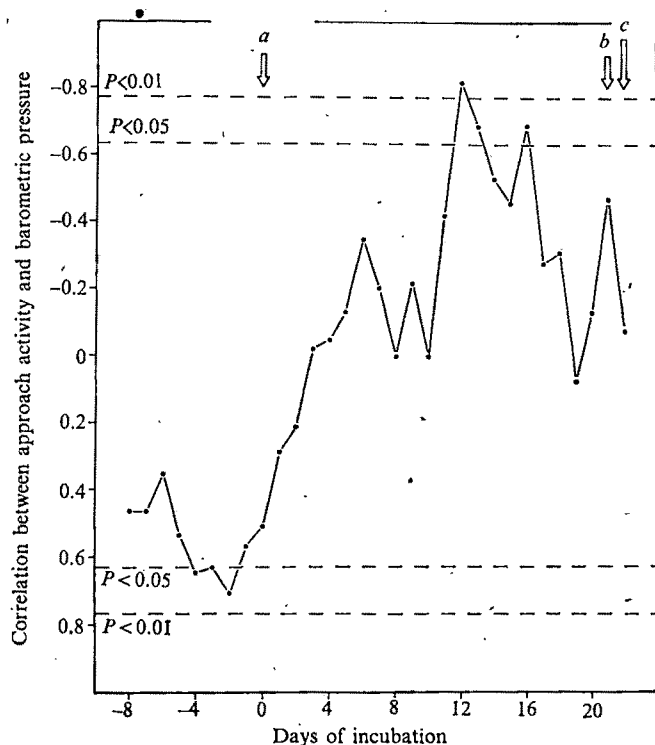


Fig. 1 The correlation between barometric pressure and the approach activity of day-old chicks plotted against the day of incubation from which pressure readings were taken. Spearman correlation coefficients are given. *a*, Onset of incubation; *b*, hatching; *c*, testing.

The chicks were placed in running wheels facing flashing, rotating lights⁶. Typically day-old chicks approach such a light after a short delay and do so with increasing vigour. They learn the characteristics of the light to which they are exposed and their social preferences are increasingly restricted to the familiar object (imprinting). In these experiments the chicks were exposed for a total of 72 min and later were given a choice between the familiar object and a novel one in an apparatus which provided a quantitative measure of each bird's preference. The principal behavioural measures examined were: latency—the time taken from the beginning of the period of exposure to the moment when approach towards the flashing light began; approach activity—the counts of approach activity in the 20 min after each chick had first started to approach; and preference for familiar—the extent to which, in a choice test, the birds preferred the light

to which they had been previously exposed. Data for each batch are shown in Table 1. Noon barometric pressure readings were obtained from a meteorological station at Oakington, Cambs. which is 4.5 km from the laboratory and 13 m lower. During the period of the experiment the maximum recorded was 1,036 mbar and the minimum 984 mbar.

Barometric pressure was most strongly associated with approach activity. Figure 1 gives coefficients for correlations, between mean approach activity and barometric pressure, plotted against the day of incubation on which pressure was recorded. On the twelfth day of incubation, the correlation was negative and highly significant ($P < 0.01$); the lower the pressure, the more active were the birds when their approach scores were measured on the first day after hatching. The correlation was positive, though not as striking ($P < 0.05$), when pressure readings were taken from a few days before the onset of incubation. While this might be important, the pattern of weather at the time of the experiments was such that barometric pressure recorded 2 d before the onset of incubation was negatively correlated with pressure 12 d after the onset (Spearman $r_s = -0.697$, $P < 0.05$). Latency was not correlated with pressure at any time nor was preference for familiar. Another weather variable, relative humidity, which might conceivably be important, was not correlated at any time with approach activity. Hatchability, the mean of which was $84.52 \pm 1.90\%$, was not correlated with pressure. The median length of incubation was, however, positively correlated with pressure on the twelfth day of incubation ($r_s = 0.707$, $P < 0.05$); the higher the pressure, the longer the time taken from the onset of incubation to the peak period of the hatch. The length of incubation might have influenced activity since all the birds were tested at the same time in relation to the onset of incubation, and post-hatch age and approach activity are positively correlated ($r_s = 0.632$, $P < 0.05$). The activity of the chick embryos, however, could easily have affected their hatching behaviour. If the embryos were sluggish they would be expected to take longer over the highly energetic process of hatching. It seems likely that this was the case as the correlation between pressure and approach activity was stronger. But the matter can only be settled by direct experiment.

One qualification that must be made is that when a large number of correlation coefficients are calculated, a certain proportion of them would be expected to reach the arbitrary levels of statistical significance on a chance basis. Nevertheless it does seem probable that a causal link existed between atmospheric pressure in the second half of incubation and the chicks' subsequent activity. If so, what could the link have been? Possibly the slight effects of pressure on the concentrations of oxygen and carbon dioxide dissolved in the blood are

TABLE 1 Behavioural measures from ten different batches of domestic chicks.

Date of hatching (1973)	Number tested	Post-hatch age at experiment (h)	Latency (s)	Approach activity (Counts/20 min)	Preference for familiar (cm)
March 7	10	22.2 \pm 0.3	174.8(76.9–817.7)	207.3 \pm 54.8	22.9 \pm 13.0
March 20	10	16.2 \pm 0.5	79.1(20.7–140.2)	164.8 \pm 39.2	7.1 \pm 17.9
March 22	12	18.9 \pm 0.2	203.7(60.2–346.9)	291.8 \pm 66.2	13.6 \pm 14.3
April 3	8	22.0 \pm 0.4	110.1(76.1–389.0)	368.1 \pm 76.9	6.8 \pm 16.7
April 10	10	22.5 \pm 0.2	192.9(116.5–354.7)	325.5 \pm 73.1	18.5 \pm 18.0
May 1	11	20.5 \pm 0.5	176.1(30.6–268.6)	232.3 \pm 37.5	9.5 \pm 16.5
May 8	10	22.2 \pm 0.2	337.4(218.0–683.9)	324.0 \pm 69.8	16.8 \pm 14.7
May 15	12	22.3 \pm 0.2	185.6(100.0–416.1)	365.0 \pm 55.0	2.8 \pm 8.4
May 17	12	21.7 \pm 0.2	219.0(162.9–398.8)	299.6 \pm 60.2	–9.8 \pm 13.0
May 22	11	20.2 \pm 0.6	112.0(31.2–192.8)	216.9 \pm 47.8	–2.2 \pm 12.7

Means and standard errors are given except for latency for which medians and interquartile ranges are used. In measuring approach activity four counts were obtained for each revolution of the running wheel. The preference for the familiar was the maximum distance travelled from the midpoint between the familiar light and a novel one. Each chick was placed in a specially geared wheel in which the bird's movements carried it away from the object which it tried to approach. When a chick has been carried sufficiently close to the less preferred light, it usually turned around and attempted to approach that one⁷. A negative score means that a chick attempted to approach the novel stimulus.

critical at a particular stage in the development of the chicks' central nervous system. Hatchability is reduced when eggs are incubated at high altitudes and may be improved by increasing the partial pressure of oxygen⁷. The range of barometric pressure in the experiment reported here, however, corresponded to a difference in altitude of less than 450 m and, of course, pressure fluctuated. Furthermore no association was found between pressure and hatchability. The results certainly suggest that it would be worthwhile examining experimentally the effects of small transient changes in pressure on measures which are more sensitive than the percentage of eggs that hatch. Whatever the outcome of such analysis, the control of pressure may remove one of the irksome sources of differences between batches of chicks.

Behavioural and biochemical details of the experiment reported here will be published elsewhere. I thank Miss J. B. Jaekel for assistance and Drs. G. Horn, and R. Oppenheim, and Miss M. A. Vince for their comments on this manuscript. The work was supported by a grant from the Science Research Council.

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Nickel accumulation by *Hybanthus floribundus*

ACCUMULATOR plants are known for many naturally-occurring chemical elements¹. The term 'accumulator plant' is used here to describe plants which have dry-weight elemental concentrations greater than either the associated substrate or 'normal' plants. Much work has centred on copper², zinc³ and selenium⁴ accumulators but little attempt has been made to explain the rationale of elemental accumulation. It is usually considered a 'tolerance' mechanism⁵, and only recently has the functional or essential role of these elements been suggested⁶.

Hybanthus floribundus is an Australian shrub species which accumulates nickel and cobalt with modal levels of 5,000 p.p.m. and 100 p.p.m. respectively, in leaf tissue⁷. Two zinc accumulating *Hybanthus* varieties have also been discovered in the northern savanna zone of Australia.

H. floribundus embraces several sub-species or ecotypes, (to be fully described elsewhere), and has an insular or disjunctive distribution across the southern part of Australia. The three nickel accumulating ecotypes, however, are re-

stricted to a broad belt in Western Australia, which extends from Ravensthorpe near the southern coast, to Agnew, 450 miles to the north. One of these varieties (*H. floribundus* ssp. *curvifolius*) is the subject of detailed distribution and biogeochemical studies in the central part of the belt⁸. The habitat of this shrub is characterised by nickeliferous soils which are defined here by nickel levels in excess of 400 p.p.m. (water-soluble nickel varies around 10 p.p.m.) These soils are often lateritised and result in low calcium levels (less than 4,000 p.p.m.) and a pH range from 5.0 to 6.9. The associated plant species are xeromorphic *Acacia* and *Eucalyptus* trees, and *Eremophila* shrubs. The *Hybanthus* shrubs are conspicuous by their apparent lack of xeromorphic adaptation to a climate which becomes increasingly arid towards the continental interior. This feature has been noted by others⁹.

TABLE 1 Nickel concentrations in soil and various parts of *H. floribundus*

Entire plant	Nickel (p.p.m.)	
	Dry weight	Ash weight
Whole leaf	6,542	130,000
Photosynthetic stem	5,490	132,000
Lower woody stem	3,806	125,000
Peeled root	415	12,800
Root bark	221	4,200
Soil	770	780
Aerial organs		
Outer leaf tip	4,869	98,790
Petiole half of leaf	4,023	82,540
Photosynthetic stem	1,179	31,300
Hard inner wood	502	55,900
Soft outer wood	1,269	34,580
Trunk bark	1,653	26,690
Soil	620	630

Nickel concentrations in the various 'insular' populations of *H. floribundus* were generally observed to decrease southwards (from 8,000 to 1,000 p.p.m.) as the annual rainfall increased from 7 inches to more than 30 inches. These 'ecotypes' can be recognised sometimes on morphology alone, and always by the use of organic (amino acids) and inorganic chemotaxonomy. The principal discriminators are N-acetyl ornithine, nickel and cobalt⁹. A study of intraplant distributions for nickel and other significant elements, shows that nickel concentrations increase from the roots through the rootstock, into the stems and reach maxima towards the leaf tips (Table 1). High nickel concentrations are also seen in seed capsules (1,500 p.p.m.), viable seeds (2,000 p.p.m.) and flowers. The mean concentration of nickel and cobalt in flowers (47 samples) is 5,003 p.p.m. and 8.7 p.p.m. respectively, whereas corresponding leaf concentrations are 3,665 p.p.m. and 24.8 p.p.m. It is evident that nickel is not excluded from reproductive tissue.

The maximum nickel concentration recorded to date is 1.6% (26% nickel in ash) in mature leaf tissue and this is in agreement with Cole⁸. Other elemental concentrations in leaf tissue, given as the mean of 217 samples in p.p.m. dry weight are: chromium 3, copper 5, cobalt 20, zinc 30, manganese 100, iron 370, sodium 1,100, magnesium 3,320, calcium 3,920 and potassium 8,000. Conventional atomic absorption was used to monitor the various elements. At these concentrations it was possible to measure nickel accurately in leaf segments weighing as little as 1 mg; mature leaves typically weighed 7 mg.

The Pearson product moment correlation coefficient (*r*) indicated several significant interelement relationships (Table 2). From those which are significant, it is evident that calcium is the only element to correlate strongly with nickel. This highly significant inverse relationship is observed in

TABLE 2 Interelement correlations (r) in *H. floribundus*

	Leaf Tissue (74 samples)							
	Mg	Ca	Fe	Mn	Zn	Cr	Co	Cu
Ni	0.0163	-0.4504*	-0.0240	0.1815	0.2411	-0.1446	0.2579	-0.0043
Cu	-0.0618	-0.1903	0.3621*	0.1885	0.2187	-0.0077	-0.1192	
Co	0.2674	-0.0959	-0.0353	0.4085*	-0.1150	0.1102		
Cr	-0.0305	0.0486	0.5633*	0.2260	-0.0205			
Zn	0.0238	-0.4097*	0.0332	0.1744				
Mn	0.1528	0.1792	0.1007					
Fe	-0.0833	-0.2216						
Ca	-0.1732							
	Twig tissue (72 samples)							
	Mg	Ca	Fe	Mn	Zn	Cr	Co	Cu
Ni	0.1959	-0.6297*	-0.1025	0.1022	0.1241	-0.0915	0.0811	0.1037
Cu	-0.2055	-0.2116	0.0813	0.1911	0.2250	0.1306	-0.1889	
Co	0.2981	-0.1500	0.3807*	0.1041	-0.2140	0.3074*		
Cr	-0.1313	-0.0300	0.6699*	0.3859*	0.1345			
Zn	-0.0509	0.0408	0.1580	0.2251				
Mn	-0.0293	0.1801	0.3407*					
Fe	0.0301	-0.0660						
Ca	-0.1130							

* Correlation significant, $P < 0.01$.

leaf, stem and wood tissue. Calcium was not measured in flowers but three significant correlations were observed, namely, nickel-manganese (negative), nickel-cobalt and cobalt-manganese. The effect of the inverse nickel-calcium relationship is to maintain the nickel plus calcium concentration at near constant levels in each of the three tissues examined. This suggests it is a substitution mechanism, and is supported by the recent findings of nickel in pectinates⁸.

More detailed studies, involving leaf tissue, indicate that nickel exists *in vivo* as a small (molecular weight less than 250) water-soluble cationic compound in agreement with a previous report¹. Histochemical staining (with dimethyl glyoxime) demonstrated a remarkable concentration of nickel in the epidermal layer which has been described elsewhere^{10,11}. The subcellular localisation of nickel was indicated to be at or near the epidermal cell walls, by means of an electron probe microanalyser¹². Leaching experiments were carried out in the glasshouse. These showed that simulated summer rain removed insignificant amounts (<0.5%) of nickel from aerial tissues, in spite of the epidermal concentration.

The accumulation of nickel in epidermal tissue of *H. floribundus* has been clearly demonstrated, but there is no evidence for a disposal mechanism. The leaves are perennial and nickel is not leached from them in significant amounts. This then raises the question, is nickel required by the plant? The essential role of nickel in *H. floribundus* was proposed⁷ on the basis of a statistical analysis of analytical data. Moore¹³ however commented that 'a physiological requirement for nickel' is difficult to explain. Cole⁸ listed the following alternatives to explain the causes of nickel accumulation by *H. floribundus*; (1) internal stress as the plant adjusts to more arid areas, (2) unrestricted transpiration and (3) a requirement for large quantities of nickel. A further suggestion¹³ is for localised selection pressure as the causal factor. There are, however, several features which can be merged to provide a hypothesis for nickel accumulation by *H. floribundus*. It is surely more than coincidence that the sole species in this arid region which does not display any apparent xeromorphic adaptation is also the only nickel accumulator. The regional correlation of increasing nickel concentrations with decreasing rainfall has been mentioned and I suggest that epidermal accumulation of nickel (where concentrations probably approach 5% or more, fresh weight) reduces cuticular transpiration and that in fact, nickel accumulation by *H. floribundus* is a xeromorphic adaptation. The origins of this idea are not new (see refs in ref. 14).

The need for nickel can account for the restriction of *H. floribundus* to nickeliferous soils in this arid environment. The present day disjunctive range of this species is the remnant of a former widespread distribution^{15,16} which retreated southwards with the onset of aridity. It is possible that the insular populations survived because they had evolved the ability to accumulate nickel, no doubt aided by polyploidy. Metal accumulation as a xeromorphic adaptation, may have application to accumulator plants in relatively arid regions elsewhere, and in particular to *Becium homblei*, the copper accumulator¹⁷ of south central Africa. Field experiments are now being set up in an attempt to establish the validity of this model. An initial approach will be to quantify the effect of nickel, cobalt and calcium on the drought resistance of *H. floribundus*.

I thank Dr R. R. Brooks and Professor P. J. Peterson for supervising early parts of this work, and also Drs G. A. Challis, P. F. Reay, A. Craig, W. D. Bennett and E. S. T. O'Driscoll for assistance.

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book reviews

Coral, the living rock

Biology and Geology of Coral Reefs. Edited by O. A. Jones and B. Endean. Vol. 1 (Geology 1): pp. xvi+410; vol. 2 (Biology 1): pp. xxi+480. (Academic: New York and London, November 1973 and January 1974.) Vol. 1: \$28; vol. 2: \$42.50; £20.40.

THE appearance of these two volumes, to be followed by a like number, indicates the present interest in coral reefs. The great controversies of the last century concerning their origin, unresolved by the outcome of expeditions to Funafuti, were succeeded by a period of greater interest in living corals including taxonomy and the vexed significance of growth forms, work associated with the names of Mayor and Vaughan in the United States, of Stanley Gardiner in Britain and of Dutch workers in the then Netherlands Indies with the lone enquiring figure of Wood-Jones at Cocos-Keeling.

It was on the falling crest of this wave that in 1928 I took the Great Barrier Reef Expedition to Australia to study reefs as living systems but the only follow up came from the Tropical Biological Station established by the Japanese at Koror in the Palau Isles in 1935 and later destroyed in the war.

The present wave of interest was generated by mighty forces. In 1945 the United States Government decided to test atomic weapons at Bikini Atoll in the Marshall Isles. 'Operation Crossroads' in July 1946 was followed by other explosions until testing ceased in 1958. Accompanying operations involved major oceanographic surveys with wide ranging geological and geographical studies including the deep drillings on Eniwetok which, at long last, ended the controversy in Darwin's favour. Most detailed analyses of coral formations included taxonomy and ecology of corals.

But although attempts were made to estimate productivity, there was a singular lack of what may be described as intimate contact with living corals. This came with the work of T. F. Goreau in the Caribbean. Starting with calcification which he studied experimentally *in situ* below the surface, he proceeded with ecological analyses of coral formations and also revealed the unexpected richness of the Atlantic coral fauna. The greatest blow that coral reef studies have suffered was his premature death in 1970.

The editors of these volumes which summarise much of what has been achieved are connected with the Great Barrier Reef Committee at Brisbane which maintains an active centre for teaching and research on Heron Island in the Capricorn Group. When completed by the publication of two further volumes, the entire work will contain 46 contributions by 41 authors from 6 different countries. Unfortunately there is no indication of the contents of these future volumes so that one cannot judge how complete will be the final coverage.

There is little arrangement of chapters and no cross references between them; each must be read in isolation; however one knows the difficulties inherent in such composite works. The first, geological, volume which is essentially descriptive, contains an excellent account of Caribbean reefs by J. D. Milliman. His comparisons between these and Indo-Pacific reefs are illuminating and his description of sand cays as "slowly moving sand waves" is apt. There is no one so well qualified as Harry Ladd to write about Bikini and Eniwetok Atolls and everything affecting corals that occurred there. All will be grateful for this comprehensive statement.

The extensive recent work on reefs in the Indian Ocean, including Aldabra, which was covered by the symposium in 1971 is well summarised by David Stoddart. The reefs of French Polynesia (The Marquesas, Tuamotus, Society Islands, and the Austral Archipelago) with those of New Caledonia are described by J. P. Chevalier. F. W. Whitehouse deals with the reefs off New Guinea but strangely without illustrations: the one map referred to was certainly not in the copy I have received. Not surprisingly the volume ends with five chapters devoted to various aspects of the Great Barrier Reef, from the nature of its waters to geomorphology, structural and tectonic factors and sediments. There is a mass of useful information here—asking to be coordinated some day—although the maps illustrating distribution of sediments, some of them appearing isolated at the end of the book, are very difficult to understand; colour should have been used.

Much is made in the biological volume of microbial ecology and the pos-

sible—for it is as yet no more—significance of the microflora in coral nutrition. In a later chapter on marine antibiotics, interesting enough in itself, there seems to be no specific reference to scleractinian corals, only to gorgonids which are now, at any rate in the Caribbean, assuming unexpected significance as a source of prostoglandins.

Leonard Muscatine gives an expectedly judicial survey of the nutritional problem in hematyphic corals. But his extensive references to our work in 1928/29 is clear indication of how little, apart from studies on the zooxanthellae, to which he has contributed so notably, has since been done. J. H. Connell's chapter on coral ecology is also to be commended. He stresses the significance of interactions between coral colonies, a further indication (so badly needed) that, despite the immobile masses of lime they form, corals are living animals.

Peter Glynn contributes a long descriptive account of the ecology of coral reefs in the Caribbean which can usefully be read with Milliman's chapter in the other volume. G. J. Bakus provides what I take to be the fullest review to date of knowledge about tropical holothurians, so numerous in species and so ubiquitous on sandy areas of reefs. The last two chapters deal with that most destructive echinoderm, *Acanthaster planci*, adding to the rich literature on this now notorious, but biologically so interesting, starfish.

These volumes contain a wide range of information, the chapters inevitably varying widely in degree of relevance and mode of approach. They will be of real value to all engaged in aspects of coral reef research although they are hardly likely to draw anyone to the subject. They lack the breath of recent research which permeates the pages of the just completed "Coral Reef Project. Papers in Honor of Dr Thomas F. Goreau 1924–1970" (*Bull. mar. Sci.*, Miami, vol. 23) which should be seen by all who are interested in the volumes under review. The two further volumes will soon, one hopes, be available. The editors somewhat strangely dedicate the work in part to themselves but, more significantly, add the name of H. C. Richards, at one time Professor of Geology at Brisbane, to whom I gladly offer a personal tribute.

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Studies on Excitation and Inhibition in the Retina

EDITED BY F. RATLIFF

April 1974: 688 pages: £8.50

For nearly half a century, Nobel Laureate H. Keffer Hartline has conducted research on vision and the retina. His studies have elucidated numerous fundamental principles of retinal physiology which, over the years, have provided the foundations for many advances in the neurophysiology of vision. This collection of papers from Professor Hartline's laboratory, together with the specially written introductory sections, provide for the first time a full account of the development of this subject, and show how Hartline's particular mathematical-experimental approach is being adopted widely in the analysis of complex neural networks.

Plant Viruses

K. M. SMITH F.R.S.

Fifth edition, March 1974: 224 pages: £3.30 Science Paperback £1.30

This new edition has been expanded to cover the present knowledge on the whole field of plant viruses. So far as it is possible in a book of this size, all the latest developments in the subject are included, such as the assembly of some virus particles, the discovery of the 'viroids', the latest information on the transmission of viruses by fungi and the most recent work in the way that viruses affect the fungi and also the algae.

Telecommunications

J. BROWN

Second edition, 1974: 400 pages £5.50 Science Paperback £3.00

A Review of the Principles of Electrical and Electronic Engineering

To be published in four volumes

EDITED BY L. SOLYMAR

Volume 1: Principles of Heavy Current Engineering

February 1974: 124 pages: £1.65.

Volume 2: From Circuits to Computers

February 1974: 192 pages: £2.50

Further details on these titles and a list of stockists available from the publishers on request.

Colonial animals

Animal Colonies: Development and Function Through Time. Edited by Richard S. Boardman, Alan H. Cheetham, and William A. Oliver, jun. Pp. xii+603. (Dowden, Hutchinson and Ross: Stroudberg, Penn.; Wiley: Chichester; December 1973.) £17.50.

THIS book, the outcome of a symposium held in Washington in 1971, consists of a series of papers which attempt to analyse the basic nature of metazoan colonies and to provide new information on the development and function of key groups of colonial animals, with emphasis on those with good fossil records. The various papers illustrate two common themes as regards the definition of coloniality: members of the colony must be physically connected and they must have common ancestry through a sexual reproduction; social insects and certain 'colonial' forms are therefore excluded. The assumption of genetic constancy allows a direct evaluation of other factors controlling morphological and physiological differences within the colony, such as ontogeny, polymorphism and environment.

Attention is restricted to four groups of animals, the coelenterates, bryozoans, graptolites and sponges (whose interpretation as solitary or colonial animals remains controversial). The treatment of these groups, however, can hardly be described as balanced. Thus the bryozoans cover 42% of the papers and 55% of the pages whereas the unfortunate sponges get only 11% of the papers and 6% of the pages.

With regard to the individual contributions of authors, it is difficult to make generalisations, because they range from lengthy, comprehensive reviews to short morphological, physiological or ecological accounts of individual species or even, in a couple of cases, to one page abstracts. Most of the material can only be adequately evaluated by a specialist in the particular groups concerned, but several papers stand out as of more general interest. For instance, Coates and Oliver examine the kinds and levels of integration in fossil and living zoantharian corals and discuss the evolution of coloniality in relation to the formation of reefs. Boardman and Cheetham, in the longest paper in the volume, evaluate the pattern of increasing structural and functional integration of bryozoans through geological time, and infer the operation of mosaic evolution. Schopf propounds and confirms to his satisfaction a model relating the degree of polymorphism in bryozoans to stability of the environment. Urbanek discusses the organisation of graptolite colonies, which are interpreted in terms of physiological gradients and genetics and in

the broader context of evolution. Although the book as a whole has a palaeontological bias there is a full coverage of living organisms and some papers deal exclusively with these.

Illustrations are abundant, with 140 line drawings and more than 200 photographs, and the production is good. The book can be recommended to all professional palaeontologists and zoologists interested in colonial animals.

A. HALLAM

Iron to carry electrons

Iron-Sulfur Proteins. Vol. 1: Biological Properties; vol. 2: Molecular Properties. Edited by Walter Lovenberg. Vol. 1: pp. xiii+385; vol. 2: pp. xiii+343. (Molecular Biology: An International Series of Monographs and Textbooks.) (Academic: New York and London, November 1973 and January 1974.) Vol. 1: \$33; vol. 2: \$29.

THE excitements and developments during barely two decades of research on iron-sulphur proteins make compulsive reading for those in the field and possibly to those who are entering it—and perhaps also to students who want to find out how research has been pursued and what knowledge has been accumulated now (or rather, about two years ago). The name iron-sulphur proteins applies to iron proteins in which the iron is liganded to sulphur—it is non-haem iron. The iron-sulphur proteins seem to be ubiquitous from the "primitive", obligate, anaerobic bacteria to higher plants and animals. They mainly function as electron carriers at very low redox potentials (although this can be altered) in most of the principle reactions sustaining life such as photosynthesis, nitrogen fixation and electron transport in subcellular membrane systems.

Volume 1 contains an excellent chapter on nomenclature and history by H. Beinert which puts the field in perspective and shows how an apparently new field has been based on unrelated endeavours in three fields: photoreactions in chloroplasts, previously unaccounted for protein-bound iron in mammalian systems, and nitrogen fixation in bacteria. Now that non-destructive and specific methods of analysis such as ESR and ENDOR (with rapid freezing techniques), NMR, and Mössbauer spectroscopy are available for studying the iron-sulphur proteins (which are much more labile than, for example, cytochromes) their structures and roles are being reported frequently and the field is spreading rapidly into many aspects of biochemistry and molecular biology.

The other nine chapters of volume 1 deal with the properties and roles of

iron-sulphur proteins in diverse reactions of bacteria, nitrogen fixation (where model compounds with biological activity are being extensively pursued), photosynthesis, carbon fixation, mono-oxygenase reactions, hydrocarbon hydroxylations, steroid hydroxylation in adrenals, and flavoprotein dehydrogenases and hydroxylases.

The wealth of information now available on iron-sulphur proteins and their roles in the above types of reactions is very well presented by the authors and well edited by Lovenberg to minimise overlap. There is a comprehensive subject and author index.

For the second volume, suffice it to quote the editor's preface: it "provides an in-depth analysis of the chemical and physical properties of many of the iron-sulphur proteins. Particular emphasis is placed on the theory and use of physicochemical techniques in the study of metalloproteins".

This volume thus provides the important companion to the first where biological properties were discussed. The importance of the biological integrity of the protein when its physicochemical properties are being investigated is rightly stressed, as this point has often been neglected in the past when physical techniques have been applied to biological compounds. This is especially important with the generally quite labile active centre of iron-sulphur proteins.

There are fascinating sections on the evolution and genetics of iron-sulphur proteins, how to build up the X-ray structure of rubredoxin, the theory and practise of EPR and ENDOR, and many comprehensive tables of useful data.

It is such a pity that it inevitably takes so long for a book to reach the desks of researchers in such rapidly developing fields; but there is now a comprehensive summary by specific workers in the field up to 1972 (addenda have been added to a number of chapters in both volumes)—and one must be thankful for this! The cost of the two volumes is very high but I hope that this will not deter anyone from having the books constantly at hand whenever referring to iron-sulphur proteins.

D. O. HALL

The tektite mystery

Tektites. Edited by Virgil E. Barnes and Mildred A. Barnes. Pp. xv+445. (Benchmark papers in Geology.) (Downden, Hutchinson and Ross: Stroudberg, Penn.; Wiley: Chichester, 1973.) £10.

As if problems recognised to be geological were not numerous enough, tektites seem to present an entirely separate mystery. Strewn fields of these strange objects are known at eight restricted

sites scattered over the globe. They mostly weigh tens of grams, and consist of highly refractory compounds, about 80% silica for example. The oldest are the bediasites at about 40 million years, and the australites youngest at under a million years. They bear little discoverable relation to their surroundings, yet they are found practically where they must have originally fallen from the sky.

Held together by surface tension just before they finally come through the high atmosphere, the tektites are there distorted into numerous aerodynamic forms: some australites starting quite spherical ablate to characteristic button-like shapes, which can and have been precisely reproduced in wind-tunnels. Remarkable indeed is the property that within every cubic centimetre of a tektite are embedded hundreds of micron-sized intrusions, while minute occluded gas bubbles are at pressures of order only a thousandth of an atmosphere.

This book consists of 45 individual papers, grouped by topics each with introductory editorial comments, and provides a volume of fascinating interest. It must cover everything known and conjectured about tektites, except perhaps a satisfactory answer to the great problem of their origin. Possible relevance to solving the re-entry problem (for spacecraft) intensified their study at that time, and for a decade a source from meteoritic lunar impacts was widely supported as the most likely answer. But the moon has since been more closely examined, with nothing much hopeful for tektites found thereon, and a terrestrial source now seems inescapable.

But they cannot result immediately from impact, and the only possibility would seem that they represent a far more remote end product of terrestrial meteoritic impacts, of sufficient violence to gasify huge amounts of surface rocks and project a vigorously expanding cloud from the resulting explosion. In cooling, the most refractory substances would condense out, and sprayed in all directions, their trajectories round the Earth would converge again to form a heated and melted accretion stream descending at the antipodal region. Fragmenting under terrestrial acceleration into solidifying droplets, these would continue to be bombarded for a time by further tiny solid particles converging to the stream.

All this may seem highly improbable, but it would explain why no tektites are found in the whole Soviet Union (one sixth the land surface) since this lies opposite much of the watery Pacific, whereas the known fields are all effectively antipodal to land masses. And if this (very briefly) is not how they were formed, one can only ask what other mechanism can be suggested?

R. A. LYTTLETON

Stave off starvation

Nutritive Value of Triticale Protein (and the Proteins of Wheat and Rye). By Joseph H. Hulse and Evangeline M. Laing. Pp. 183. (International Development Research Center: Ottawa, 1974. \$7.50.

THOSE of us who are concerned about the long-range prospects for world food supplies have for many years been grumbling that we depend for almost all our food on plant species selected by primitive man—or more probably woman. The first break with this unseemly dependence on the past comes, not from the domestication of a new wild species, but from the creation of a new species. This is triticale: a hybrid of wheat and rye (*Triticum x Secale*). Ostensibly, this is a book about it. Unfortunately, the authors have spread themselves over the whole subject of the properties and manner of use of wheat and rye, and the results of adding supplements such as amino acids, soya, fish and microbial products to them. Triticale gets rather lost; less than a third of the book deals specifically with it. Mainly, the authors have simply copied out their index cards after rough classification. Incompatible conclusions appear side by side without comment, though some reconciliation is attempted in a 17-page introductory survey.

Triticale can be made by crossing several subspecies of wheat and rye; it is therefore a genus rather than a species. Sterile crosses were made in 1875, colchicine-induced polyploidy made them partially fertile in 1937. Since then many fully fertile strains have been produced, compared and standardised. In comparable conditions the better strains seem to give larger yields than wheat; an experiment is quoted in which triticale yielded 8.3 and wheat 7.2 tons per hectare. As with wheat and rye, the protein content of the grain varies with cultural conditions, but triticale seems usually to surpass the other two. The amino acid analyses quoted are by no means concordant. Before the statement that triticale contains more lysine than wheat is accepted, we should be told how many analyses were done on each sample, how many samples from the same crop were taken, and how amino acid composition is affected by cultural conditions. Amino acid analysis is not such a foolproof process as it is often assumed to be.

Many of the properties of triticale are intermediate between those of its parents. It contains less resorcinol than rye and is less susceptible to ergot, but it is not as suitable for bread-making as wheat though a mixture of flours is satisfactory. There seems to be no evidence on the phytic acid or trypsin

inhibitors in triticale; in spite of this, the authors devote four pages to these components of wheat and rye. Triticale is a new crop on which much more research and development are needed. Its superiority to its parents is not yet unequivocally established but is probable. It seems clear that triticale is potentially a valuable crop: someone ought to write a book specifically about it.

N. W. PIRIE

Polar humans

Polar Human Biology. (Proceedings of the SCAR/IUPS/IUBS Symposium on Human Biology and Medicine in the Antarctic.) Edited by O. G. Edholm and E. K. E. Gunderson. Pp. xii+443. (Heinemann: London, November 1973.) £6.00.

THE symposium from which this book resulted took place in Cambridge during September, 1972. The editors are to be congratulated on producing it within a reasonable time and the publishers have provided an attractive format and a price which is not excessive. Certainly, the book deserves to be read widely, for it describes the state of the art of research into polar human biology and medicine at a time when great advances have been and are being made.

The book falls naturally into five sections. An initial series of reviews of the individual contributions to Antarctic research by the several countries concerned is followed by two papers on dental problems and a larger section on virological and immunological research. Next follow a group of papers on various aspects of thermal adaptation, activity patterns and circadian rhythms. The book ends with a large section devoted to psychological and social problems of life under the severe conditions which constitute the polar environment.

As might be expected with such a large range of subjects, the treatment is very uneven. Several of the papers are either anecdotal or present largely unanalysed sick-report data. Though of practical interest they seem out of place here. On the other hand a few papers seem destined to become classics in the study of man at extreme latitudes. In particular the two papers of Shephard and his collaborators Godin and Rode seem to me to combine all that is excellent in a multidisciplinary study of the response of the Eskimo to his rapidly changing pattern of life. Roger's paper on cold adaptation in members of the Commonwealth Trans-Antarctic Expedition of 1958 is equally admirable—indeed, perhaps more so, since the conditions of work must have been much more severe. His conclusion—that general biological adaptation to severe cold does not occur—is interesting to say the least!

The importance of psychological and cultural factors in polar adaptation is rightly stressed. Much of it is of an applied nature, in particular for personal selection, and several of the papers reflect this interest. Again, though, one wonders if this book is the right vehicle for such reports.

Though the book contains much that is good and some things which are really excellent, it is impossible to escape the general conclusion that the international organisation of polar (and particularly Antarctic) research in human biology is in a pretty anarchic state. A good many papers show considerable areas of overlap of topic and similarity of methodology and it seems sad that the impetus of the International Biological Programme towards cooperation and standardisation seems to have petered out in the far south. I hope, though, that this book will be a spur to the greater integration of research. If this happens the enormous devotion and self sacrifice of many of the scientists, working often in an environment as lethal as any in the world, will be fully rewarded.

E. J. CLEGG

Biological information

Elementary Principles of Probability and Information. By R. F. Wrighton. Pp. viii+91. (Academic: London and New York, January 1974.) £1.95; \$5.50.

THE author is primarily concerned with the applications of statistical inference and information theory to the biological sciences. He complains that biologists are 'currently witnessing a resurgence of anthropomorphism as information notions percolate into biological enquiry conceived at the molecular level'. He foresees that present macro-theories, derived essentially from 'purely subjective interpretations of probability' and applied to information processes of macro-systems in which it is always possible to distinguish between 'the label and the labelled', will break down at or near the molecular level.

At first sight, his programme seems to imply a direct though difficult reduction of biology to physics. But the programme is even more fundamental than that. In his last chapter, noting that anthropomorphic heresies, in the form of traces of communication notions, pervade both quantum and relativity theories, the author looks forward to a 'complete fusion of information theory with quantum theory rather than to any less intimate association'. He is therefore claiming that 'the much closer physical analysis he seeks of biological processes could solve some of the epistemological problems of modern physics. That is an ambitious programme. Is it possible for man to establish a science from which all anthropomorphic residues

Corrosion of metals research 1924-1968

This book, written in a semi-popular style, by Dr Wormwell of the National Physical Laboratory, is intended for people with an interest in corrosion and its prevention. It is a modest contribution to the history of government science and offers an example of what can be achieved by a long-maintained team working in one broad area of endeavour.

£1.65 (£1.72)

A code of practice for the detailed statement of accuracy

The main purpose of this Code of Practice, prepared by the National Physical Laboratory, is to put forward recommendations as to how uncertainty can be expressed so as to avoid ambiguity, and discusses some of the ways in which estimates of uncertainty can be derived from individual measurements.

£1.05 (£1.10)

Prices in brackets include postage

Government publications can be bought from the Government bookshops in London (post orders to PO Box 569, SE1 9NH), Edinburgh, Cardiff, Belfast, Manchester, Birmingham and Bristol, or through agents and booksellers. The Bookseller section of Yellow Pages gives your nearest stockist.

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are completely eliminated? If so, then at least we have to begin by identifying those residues that can be discerned and considering what might be done about them. And that is what the author attempts to do.

Unfortunately, the reader finds himself embarking on this fundamental programme without being clearly informed about where he is being led. The seven chapters discuss familiar topics in an unfamiliar way—randomness, the basic Shannon model, noise, inverse problems in probability, sampling techniques, and so on. These topics, dealt with in terms of elementary mathematics, enable the author to air the serious problems he has in mind. But it is only slowly and with much re-reading that the reader builds up any coherent picture of the author's objectives. So it is difficult to see to what readers this book is addressed. Those seeking techniques for immediate practical application will find none: those interested in the fundamental problems discussed will be misled by the title and would not expect so serious a programme to be presented in this form.

But the author's ideas are stimulating. Information concepts derived from telecommunications and other physical systems do seem to be inadequate for enquiry into the processes of 'information transfer' on which all living organisms depend. B. C. BROOKES

matters arising

Temperature cycle in the North Pacific

SIR,—I suggest that the evidence presented by Favorite and McLain¹ for trans-Pacific displacement of temperature anomalies at the ocean surface, is far from conclusive and that there is no evidence that waves progress around the North Pacific gyre.

The eastward advance of the anomalies across the Pacific Ocean from 1954 to 1960 is not immediately obvious or orderly. An explanation of the widespread warming in 1955 of the eastern Pacific between 35°N and 40°N, in terms of an eastward displacement of the maximum, implies a rate of advance of some 15 km d⁻¹. This is about three times greater than the generally accepted pattern of geostrophic flow, which is slightly more than 5 km d⁻¹. Similarly, the rate of advance of the positive anomaly from the western side to the eastern side of the ocean from 1956 to 1957 would have been some 15–20 km d⁻¹. Conversely, there was very little change from 1958 to 1960, when, according to the proposed hypothesis, the negative anomaly should have progressed eastwards to embrace the western coast of North America. Another discrepancy is the westward movement of the negative anomaly in the eastern Pacific from 1955 to 1956.

If the rate of advance of the temperature anomalies, indicated by geostrophic calculations, is slightly more than 5 km d⁻¹ and the circumference of the North Pacific gyre is some 18,000 km, then the time required for a water parcel to complete one circuit would be nearer 1 yr than the 6 yr suggested.

Suggestions that there is evidence of

the temperature cycle as far back as 1930 in the western subarctic region carry little weight in view of the statement that "it is difficult to establish normal conditions at any time or place that would allow the detection of anomalous conditions in a water column at the western side of the ocean."

A simpler explanation of the 5–6 yr cycle in sea surface temperature anomalies in the North Pacific is that heating and cooling occur *in situ*. Baur² has shown that the intensity of the solar beam undergoes a double systematic variation, amounting to 0.5%, within the 11-yr sunspot cycle, with primary maximum strength at about 0.4 of the rising phase and a secondary maximum at about 0.6 of the declining phase of solar disturbance; a sharp minimum of the solar constant occurs just before sunspot minimum, with a secondary minimum at about 0.6 of the rising phase. These variations of the solar constant have been applied to the known years of sunspot extremes from 1950 to 1968 (ref. 3). Figure 1 shows that there is a high positive correlation between sea surface temperature anomalies in the North Pacific, and fluctuations of the solar constant.

There are other well documented relationships between variations in the output of solar energy and meteorological and climatological phenomena. The height of the tropopause at Leopoldville, near the equator, rose by more than 1 km between the 1954 solar minimum and the 1957 maximum (ref. 4). This could be interpreted as resulting from a strengthening of the ascending branch of the Hadley cell associated with greater energy transfers in the atmosphere at the time of increasing output of solar radiation. In East Africa, the level of Lake Victoria was highest in 1952, 1957, and 1964, some 1–2 yr after maxima³ of the solar constant. Again, an increase in surface heating at the time of maximum solar output would lead to an increase in convection and thus to an increased tropical rainfall. In European Russia there were sharp fluctuations in the mean temperature from month to month at times when solar activity was increasing in 1956–57⁵ and in 1967–69³. The strength of the westerlies over New Zealand tended to increase about 10 months after rises of solar activity (B. N. Parker, unpublished information). More generally, large fluctuations of Baur's solar index⁶ in the extreme sunspot

cycles since about 1940 coincide with decades of increased variability of wind circulation, with falling values of indices of the strength of the zonal windstreams and with increasing variance of climatic elements in many parts of the world³. Processes by which solar activity may influence weather and climate are discussed by Lamb³.

Thus, the 5–6 yr temperature cycle in the North Pacific could possibly be caused by systematic variations of surface heating resulting from variations in the output of solar energy during the 11-year sunspot cycle. This simple pattern would be modified by ocean-atmosphere interactions and quasistationary waves, to produce regional variations in the timing of the maxima and minima. The geographical distribution of temperature anomalies at each phase of the sunspot cycle would then not be expected to be the same in each cycle. In fact the coherence evident during the 1958–60 cycle was not readily apparent during 1948–52 or 1961–67 (ref. 1). It is to be expected that the effects of these variations in the output of solar energy should also be evident in temperature anomalies in other oceans.

Yours faithfully,
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¹ Favorite, F., and McLain, D. R., *Nature*, **244**, 139 (1973).

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⁵ Khrabrov, Ju. B., *Moscow, Center. Inst. Prog., Trudy*, **71**, II (1958).

⁶ Baur, F., in *Lehrbuch der Meteorologie*, 970 (Hirzel, Leipzig, 1949).

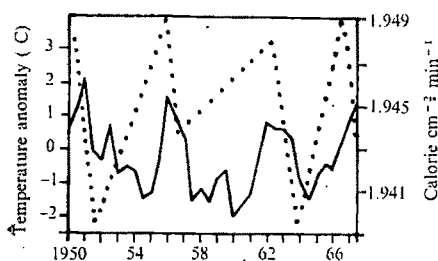


Fig. 1 — semiannual anomalies of mean sea surface temperature in Marsden square 163, quadrant 1 (40–45°N, 170–175°E) from 20 yr mean, 1948–67 (from ref. 1). Average variation of solar constant from phase to phase of the 11 yr sunspot cycle² applied to the sunspot extremes³.

Macromolecular structures for undergraduates

MEYER¹ and Feldman *et al.*² describe the use of computer displays for the three-dimensional study of macromolecular structures. They suggest that, in the near future, such systems will be available for teaching purposes. As students we should like to point out that computer graphics have disadvantages for teaching in England, and that there are now available molecular models which may be used to emphasise different aspects of structure in an immediately

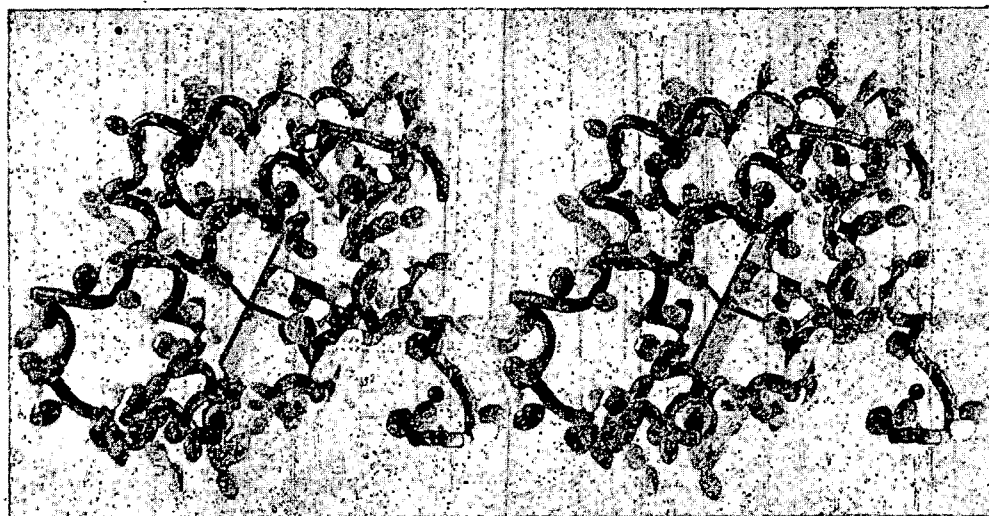


Fig. 1 Myoglobin stereopair built from Biobits model system. The helices, N- and C-terminal ends, haem pockets, types of R groups and their location on the surface or within the interior of the molecule are readily and immediately obvious.

comprehensible manner. One of these systems³ in particular makes possible the building of a complete macromolecular structure such as myoglobin within a framework 60 × 60 × 30 cm (Fig. 1) and in one to two days.

One problem in the use of computer graphics is the cost of installing and running the apparatus. Another is that students would need specialised training to use it. Models have the advantages that they are built by the student, can be handled and viewed from all sides, and are portable. Models display more than adequately for the undergraduate the features of families of macromolecules: there seems little point in listing the features of molecule after molecule in a computer display.

Biobits models for proteins and nucleic acids are available from Capital

Biotechnic Developments Ltd., 66A Churchfield Road, London W3, United Kingdom, and from Ealing Corporation, 2225 Massachusetts Avenue, Cambridge, Massachusetts 02140. With these models we have been able to build accurate scale models of myoglobin, carboxypeptidase A, LDH monomer and other proteins within a day or two of receiving the X-ray data. Indeed we have probably built and studied a wider range of proteins than any other undergraduates in England including those in the major X-ray crystallographic departments.

We appreciate the tremendous value of computer graphics: the system can, for example, overlay structures of two molecules for direct comparison in a manner which models could never do. But we hope that before any depart-

ment switches to this complex and expensive system it will reconsider the very great advantages of real models.

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¹ Meyer, E. F., *Nature*, **232**, 255-257 (1970).

² Feldman, R. J., Bacon, C. R. T., and Cohen, J. S., *Nature*, **244**, 113-117 (1973).

³ Smith, I., Smith, M. J., and Roberts, L., *J. chem. Ed.*, **47**, 302-305, (1970).

Announcements

International meetings

May 1-5, **22nd Annual Colloquium on Protides of the Biological Fluids** (XXIInd Colloquium on Protides of the Biological Fluids, Simon Stevin Instituut, Jerusalemstraat 24, B8000 Brugge, Belgium)

May 5-9, **ECBO Study Group on Ageing: Symposium on Impairment of Cellular Functions during Ageing in vivo and in vitro** (Dr E. Holečková, Czechoslovak Academy of Sciences, Institute of Physiology, Budějovická 1083, 142 20 Prague, Czechoslovakia)

May 6-8, **International Water Pollution Conference** (Conference Secretariat (EEC Amsterdam), Society of Chemical Industry, 14, Belgrave Square, London SW1X 8PS, U.K.)

May 6-10, **First International Mercury Congress** (Secretaría del Congreso, 2016 3462)

Facultad de Ciencias (Pedralbes) Barcelona 14, Spain)

May 8-10, **Minicomputers in Data Communication** (Penny Green, Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS, U.K.)

May 8-11, **Management Techniques for Metallurgists** (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N 20 9LW)

May 14, **Symposium on Cancer—The Patient and Family** (Mr P. A. Sturgess, Assistant Secretary, Marie Curie Memorial Foundation, 124 Sloan Street, London SW1X 9BP)

May 14-16, **Conference on Neurobiology of CNS Hormone Interactions** (Dr Walter E. Stumpf and Dr Lester D. Grant, 111 Swing Building, University of North Carolina, Chapel Hill, North Carolina 27514)

May 14-18, **West European Conference on Marine Technology** (The Royal In-

stitution of Naval Architects, 10 Upper Belgrave Street, London SW1X 8BQ)

May 14-22, **US-ROC Seminar on Plant Tissue and Cell Cultures** (Dr L. C. Nickell, Hawaiian Sugar Experiment Station, Hawaii)

May 15-17, **3rd International Conference on the Hydraulic Transport of Solids in Pipes** (Organising Secretary, Hydrotransport 3, BHRA Fluid Engineering, Cranfield, Bedford MK43 0A, U.K.)

May 16, **Friction, Wear and Lubrication** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

May 16-18, **Physiology of the Nephron: Mechanism and Regulation** (Secretariat, Organizing Committee, (Dr J. P. Borvalet), Unite de Recherches de Pathologie Cardiovasculaire, I.N.S.E.R.M. Hôpital Leon Bernard, 94450 Limer Brevannes, France)